DISTRIBUTION AND MORPHOLOGY OF A PHORETIC MITE, *ANOETUS HALICTONIDA* (HISTIOSTOMATIDAE) ON AN URBAN POPULATION OF *HALICTUS RUBICUNDUS* IN CENTRAL SASKATCHEWAN

A Thesis Submitted to the College of
Graduate and Postdoctoral Studies
In Partial Fulfillment of the Requirements
For the Degree of Master of Science
In the Department of Biology
University of Saskatchewan
Saskatoon, Saskatchewan, Canada
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Abstract

The ground-nesting activities of a sweat bee, *Halictus rubicundus* [Christ], and data about its commonly associated mite, now tentatively identified as *Anoetus halictonida* (Woodring, 1973), were investigated at two sites on the University of Saskatchewan campus in Saskatoon during 2014 and 2015, thereby representing the first such study of this relationship in western Canada. Plaster of Paris casting was utilized to identify and excavate 78 subterranean nests of *H. rubicundus* throughout the spring-summer of 2015, to reveal nest architecture, phenology of the bee's life stages, potential social interactions among bees, plus the types and proportions of pollen that contribute to the larval diet of this polylectic bee species. Microscopic examination of bees and brood cells revealed various life stages (eg. Tritonymphs; gravid females) of *A. halictonida*, but only deutonymphs resided on adult bees, in specific body locations according to host sex. Mite loads on adult females (96.3%) predominated on the lower surfaces of hind wings in a symmetric fashion, but also dorsally at the mesosomal-metasomal junction. Phoretic loads of deutonymphs on adult males (57.1%) averaged 1/3 that of females and instead were concentrated ventrally on the thorax and lower head. Mites residing on pre-adults (pupae and pharate bees), however, were surprisingly scattered and equal among host sexes, suggesting an eventual net mite transfer from adult male to female bees, possibly at copulation. Even the largest load of deutonymphs (167) was considered negligible (<0.5% the body weight of an adult female bee). Scanning electron microscopy revealed new information about the flexibility and microstructure of the caudal suckerplate and legs of deutonymphs of *A. halictonida*, allowing attachment to *H.* rubicundus. A brief comparison to other halictid-anoetid interactions involving *H. confusus* and *Sphecodes* in the same study area and the possibility of interspecific transfer is discussed.

Acknowledgements

I am grateful to my supervisor Dr. Art Davis, an invaluable and unwaveringly supportive mentor. Thank you to my committee members, Drs. Neil Chilton and Cedric Gillott, for their advice which was sorely needed.

Special thanks for the technical assistance provided by several very patient individuals on the University of Saskatchewan campus. Within the Biology Department Guosheng Liu provided assistance with the department SEM, whereas Marlynn Mierau, and Marlene Mahoney helped with photography. Tom Bonli of the Geology Department and Eiko Kawamura of the Western College of Veterinary Medicine assisted with the use of their departments' respective SEMs. Clint Berschiminsky of Facilities Management Division helped arrange permission to excavate on campus, and trusted me not to dig up the sprinkler system.

Identification of unknown species was provided by Drs. Sabine Banniza (University of Saskatchewan), Heather Proctor (University of Alberta), Pavel Klimov (University of Michigan), and Barry OConnor (University of Michigan).

Thanks to my labmates in the Davis lab, especially Daniel Wiens, the resident ant expert at the time.

This project owes its funding to an NSERC USRA, a Graduate Teaching Fellowship (2014-2015 academic year), and an NSERC Discovery Grant to A.R. Davis which provided part of the stipend and the general lab supplies necessary for this project. Funding was also supplied by a Margaret MacKay Scholarship.

Lastly, thanks to my Mom and Dad, my sister, and my family for their love and support.

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CHAPTER 1 - INTRODUCTION

1.1 Symbiotic relationships

Symbiotic relationships - close interactions between different species - are ubiquitous and take many forms in nature. Whereas the word symbiosis has entered common vernacular as a way of indicating two organisms in a beneficial relationship, the scientific meaning of symbiosis is much broader and includes all close associations, regardless of benefit or detriment. Indeed, symbiotic relationships are defined and classified into specific types based on whether one or both partners are harmed, helped or generally unaffected by the relationship. It should be noted that categorizing the relationships between organisms can be difficult. The relationships are points on a continuum and do not lend themselves to being neatly classified into distinct association (Parmentier and Michel 2013). As an example, parasitism and predation are very closely related, involving energy transfers between two organisms, with one of the major barriers between the two terms being whether the association is immediate (as in predation) or prolonged (parasitism). In addition, symbiotic relationships are not static and may change over time not only on an evolutionary scale, but also on a temporal level, with some phoronts (the symbiont that attaches to the host in a phoretic relationship) becoming parasites at different stages of their life. What some think of as symbiosis is more correctly termed mutualism, which as the name implies is a mutually beneficial relationship.

1.1.1 Parasitism

One of the most well-known types of symbiosis is parasitism, in which one of the participants is harmed by the association (this individual is known as the host) while the other (the parasite) benefits from the association. Often parasites are small organisms that use their host as both a food resource and as habitat. Parasites may alter physiological processes of the host and negatively affect the host if they multiply to the point where parasite load becomes too high for the host to counteract (Loreau et al. 2005). Parasites and parasitism receive widespread study and interest due to the sometimes massive impact on human health, husbandry, and wild animals. For example, the varroa mite, an infamous pest that attacks honey bees, has an alarming impact on apiculture. Mites like *Varroa* spp., and the admittedly much less destructive honey bee tracheal mites (*Acarapsis woodi*), can cause massive losses of honey bee colonies. The worst recorded loss in the U.S.A. thought to be a direct result of *Varroa jacobsoni* (but later identified as *Varroa*

destructor) was in the overwintering period of 1995-1996, when colony losses ranged from 40-80% (Doebler 2000).

1.1.2 Exploitative symbiotic relationships excluding parasitism

Several types of exploitative behaviour that are termed parasitic should be mentioned to avoid confusion with the common understanding of parasitism. Cleptoparasitism is somewhat similar to brood parasitism, in which an egg-laying parasite makes use of the parental instincts of another organism (conspecific or otherwise) to provide and care for the parasite's offspring (Zink and Lyon 2016). Cleptoparasites benefit at the cost of their hosts, without feeding on their host. Instead, these organisms take items that the hosts have gathered, such as food, instead of spending energy gathering these things themselves. In bees, a Cleptoparasite will enter the host's nest and lay an egg in a brood cell. In bees of the genus *Sphecodes* - which is composed of a majority of cleptoparasitic species - the adult female is believed to destroy the host egg. However, many cleptoparasitic bees do not destroy the host egg, instead either hiding their egg in the wall of an unfinished cell or inserting the egg through a hole the mother makes and then closes (Michener 2007).

Social parasitism is again different from what might be expected of parasites. Social parasites take advantage of existing social structures, but they themselves are not social. It can be defined as an association in which one individual exploits mechanisms of another society, and can be found in social insects like ants, although the form of social parasitism varies (Powell et al. 2014). In bees, social parasitism refers to those instances in which a female enters a nest of the social host and replaces the queen so the workers will raise the parasite's offspring (Michener 2007). Both of these parasitic relationships are distinguished from nest usurpation and robbing.

Related to parasites are parasitoids, which operate in much the same way as parasites (one benefits at the cost of the other), but have the distinct difference of eventually killing their host. Occasionally the criteria for "death" are a bit broader and parasitoids cause a figurative instead of a literal death, such as the sterilization or "reproductive death" of stylopized bees, for example, imposed by strepsipteran parasitoids, although the distinction is moot (Kathirithamby 2009). In general, when the death of the host is not desirable from the standpoint of the exploitative organism, the relationship is parasitic rather than parasitoidal (Parmentier and Michel 2013).

1.1.3 Commensalism

Other types of symbiotic associations receive little attention as they have little to no noticeable impact on quality of life or the economy. Commensalism is classified as an association in which one organism benefits, while the other organism is neither harmed nor helped by the relationship; thus, the association is neutral for the organism that does not benefit (Zapalski 2011). Describing and identifying commensalism has its own set of difficulties. It has been argued that since a neutral effect cannot be observed, it also cannot be proven and is therefore a concept that should be relegated to the theoretical and is "unfit for empirical science" (Zapalski 2011). Commensalism is sometimes used as a term for any interaction that positively affects one species and has a weak positive or negative effect on the other organism, making commensalism very difficult to isolate from other interactions (Veiga 2016). Regardless, there are some associations in nature that require the use of such terms, imprecise as they may be, to indicate that no obvious detrimental or beneficial effects are impacting the "unaffected" organism. If more data becomes available and biases the association towards mutualism or parasitism, then it can always be redefined, but at the absolute least the word serves as a general descriptive term if not as a concrete classification.

Commensalism takes multiple forms in nature; those forms that are of importance to this thesis are inquilinism and phoresy. Inquilinism occurs when a symbiont receives the benefit of shelter from the association either in the host's own home or inside the host itself (Parmentier and Michel 2013). An inquiline can be any animal that lives in the nest or dwelling place of another species, but this definition overlaps with metabiosis; metabiosis is another type of commensalism that occurs when the actions of one species modifies or creates a habitat, which is then used by another species (Veiga 2016).

1.1.3.1 Definitions of phoresy

Phoresy is a type of commensalism involving one organism, termed the phoront or phoretic, using another for dispersal. It is applied to relationships (often interspecific) in which an organism attaches to another for the purpose of dispersal (Houck and OConnor 1991). The term "phoresie" was suggested by Lesne in 1896 to indicate insects attaching to the surface of other insects for transportation, but excludes transport for the purpose of *direct* parasitization (Clausen 1976). The original intent was to refer to instances in which the host *only* serves its passenger

(phoront) as a vehicle for transport in the strictest sense (Farish and Axtell 1971). Phoresy is no longer a term used specifically to designate insects utilizing other insects. More recent definitions of phoresy have had to be refined partly due to the increase in taxa known to participate in phoretic behaviour and as new variations were discovered. In 1917, Deegener [cited by Farish and Axtell (1971)] further added to the literature on phoresy by defining "symphorium" (resembling phoresy) as one animal settling on the body surface of another animal without the occurrence of parasitism or mutualism. Deegener's definition of a commensal relationship resembling phoresy fell into disuse in favour of refining phoresy, because he also used symphorium to describe permanent associations (phoresy is generally thought of as a temporary arrangement) and associations involving sessile creatures that do not disperse in quite the same way. Since there were two very separate definitions, one indicating a temporal arrangement and the other imprecise in meaning, Farish and Axtell (1971) provided a streamlining of the definition based on what they felt was implied and intended by Lesne's original definition: phoresy is a phenomenon where one animal seeks out and attaches to the outer surface of another animal for a limited period of time, during such time the phoront ceases feeding and ontogenesis, with the attachment presumably serving the purpose of dispersal from an area unsuited for development (either of the individual or its progeny). The authors disapprove of using the word "transport" in favour of the passive "dispersal" to avoid giving the impression of directed movement as there is no guarantee of successful transport.

A related term is hyperphoresy, in which a phoront uses another phoront as a host, although examples of hyperphoronts are somewhat lacking, possibly due to the scale of organisms involved. Ostracods phoretic on amphibians themselves will sometimes play host to ciliate hyperphoronts (Sabagh et al. 2011). Phoronts themselves are often fairly small in comparison to their host and any organism using the larger phoront as a vehicle has limits on its size and mass to allow for movement.

Phoretic behaviour has been reported in several groups, such as in the phyla Annelida and Arthropoda. Among arthropods, arachnids (mites and pseudoscorpions are among the most prolific) and insects are some of the best examples, both as phoronts and phoretic hosts (Houck 2009). These arthropods take advantage of many separate hosts depending on availability and the

methods employed. This thesis will primarily investigate those associations, phoretic and otherwise, which involve arthropods.

In a discussion dealing primarily with insects that feed on other insects (Clausen 1976), the definition of phoresy was extended to include internal transport, provided the transport occurs on a non-host and is either essential to the development of the phoretic insect or at least a common occurrence. This definition is slightly different to phoresy only applying to transport of phoronts on the surface of insect bodies, as described by Lesne's original definition. The same discussion clarifies that accidental phoresy that does not involve the host being used for development is not a "true" phoretic relationship. Rarely, feeding may occur, but this feeding is considered incidental and a minor consequence of the primary objective of transport to the life cycle stage on which feeding and development normally occurs. Among insect parasites and predators, there are many examples of phoresy, with the majority of phoretic stages being either the adult female or the first-instar or primary larvae (Clausen 1976). As noted by these occurrences, the developmental cycle of some insects in exploitative relationships can involve a switch from parasitism to phoresy, as determined by need and life cycle stage. Although it could be argued that the relationship is purely parasitic and the phoretic behaviour displayed is another facet of the association rather than true phoresy, the distinction is moot.

A recent discussion by Houck (2009) expands on the concept of phoresy by outlining certain general principles, itemized below, that are consistent among phoretic organisms (although exceptions do occur):

i) The phoront (phoretic) is usually much smaller than the host;
ii) There are often multiple passengers on individual hosts and mass transit is not uncommon;
iii) The phoront does not have an effective way of independently dispersing relative to the host;
iv) Phoresy has played a role in dispersal of that phoront for a long time and evidence is present in the geological record;
v) Phoresy is a response to an ephemeral habitat degrading, or to resource limitation;
vi) Phoronts dismount when suitable habitat becomes available;

vii) Usually only one life cycle stage (either the adult, nymph or larva) of a complex life cycle participates in phoresy;
viii) Phoresy may be highly coevolved and stenoxenic (from closely related species) under certain circumstances;
ix) Phoresy may be obligate or facultative;
x) Phoronts may have very little or very extensive modification;
xi) Phoresy may be enhanced by wind;
xii) Phoresy may be continual, or seasonal, or cyclical;
xiii) Phoront and host may represent related lineages or very disparate taxa;
xiv) Phoresy has developed independently throughout the Arthropoda many times.

It was argued by Houck and OConnor (1991) that the definition proposed by Farish and Axtell must be modified when applied to the phoretic associations in certain Acari (=Acarina). Specifically, mites are often quiescent when waiting for a host and therefore the phrase "actively seek" is somewhat misleading; mites are discriminatory in regards to potential hosts, but may not be as energetic as the definition by Farish and Axtell implies (Houck and OConnor 1991). In addition, dispersal implies scattering, whereas mites often move together on hosts in large groupings, which is argued to be more akin to migration than dispersal (Houck and OConnor 1991). The operational definition proposed by Houck and OConnor (1991) in regards to mites is thus: phoresy is a phenomenon in which one organism (the phoretic in the original definition) receives an ecological or evolutionary advantage by migrating from the natal habitat while superficially attached (the phrase "superficially attached" is meant to include transport within natural host orifices and under structures that protect from the external environment) to a selected interspecific host for some portion of the individual phoretic's lifetime *and* benefit of the association is not conferred as a nutritional or developmental influence on the phoretic stage.

1.1.3.2 Aspects of phoresy

Although the definition of phoresy is somewhat constraining, there is variation in the strategies that different phoronts employ. The specific reasons and driving forces for phoresy may be different, and the methods of finding and attaching to a potential host can differ. In those phoronts that possess different life stages, the phoretic stage may be an immature or an adult.

Since phoresy has evolved multiple times, in different orders, it is not surprising that phoretic strategy can greatly differ.

1.1.3.2.1 Reasons for phoresy

Phoresy can be a highly important strategy for survival and has been described as a biological necessity for some mites (Binns 1982). Some of the major reasons for the importance of phoresy in Acari are as follows:

i) All mites lack wings and the majority are restricted in activity. In addition, they are incapable of traversing long distances.
ii) Many mites have narrow ecological limits, especially with regards to humidity.
iii) Requirements of the phoront's immature and adult stages restrict development and reproduction, such as dietary requirements.

Whereas these reasons are specifically geared towards mites, these characteristics are generally applicable to many phoronts. Phoresy compensates for disadvantages such as small size and lack of morphological adaptations, and also provides some protection from potential predators (Houck and OConnor 1991). Phoronts are often small and restricted by the distance they can travel. However, not all phoronts are restricted as such; several facultative phoronts use phoresy to disperse despite being competent flyers or travelers. An organism's requirements may change over time or the environment it currently inhabits may no longer suit its needs. An immature organism has different priorities than an adult of the same species and may need to relocate in order to reproduce once it matures.

1.1.3.2.2 Life cycle stages participating in phoresy

Parasitic and predatory insects may become phoronts at certain stages in their life cycle to facilitate dispersal of themselves or their offspring. Numerous examples were provided by Clausen (1976), although all were of two types: transport of adult female phoronts, in all cases representing the Hymenoptera; and transport of first-instar larvae, which have representatives from Coleoptera and Strepsiptera. In the first instance, adult females (formerly parasites) are attracted to the adults of the host species, specifically the females since males will have little to no contact with the next immature generation. This affinity to the adult stage occurs following development and feeding on other stages of the host, such as the eggs. Most if not all parasites

and predators with this behaviour attack hosts that lay egg masses as opposed to singular eggs, greatly increasing the chances of survival by sheer numbers. However, this relationship is thought of as facultative rather than obligatory, with the adult female parasite still capable of dispersing and locating host eggs on its own. Several hymenopteran families use this strategy, with the most prominent being Scelionidae, but including certain species of Trichogrammatidae, Torymidae, and just one species of Eulophidae (Clausen 1976). Among mites that are phoretic on halictid bees, it is either the deutonymph or the adult female stages that are the actual phoronts (Table 1.1).

1.1.3.2.3 Finding a host

Whereas some organisms maintain an ability to travel without a host, diminishing their dependence on phoresy as a way of survival, there are certain organisms that may be heavily reliant on a host for dispersal. Reliance on dispersal predisposes a particular phoront to develop some way(s) of detecting and attaching to a potential host. The practice of phoresy requires an organism to be capable of boarding and securing itself to a host, but other adaptations that allow the phoront to distinguish hosts and the suitability of a habitat will likely increase survival (Houck and OConnor 1991).

Comparing host preferences among different phoronts that operate under similar situations can reveal important factors controlling host searching and suitability. For example, certain organisms that live in bromeliads require transportation from one plant to another, since the habitat the plant provides is transient, and some of the local inhabitants lack the means to travel. Bromeliads play host to aquatic organisms such as annelids and ostracods, which may use alternate cues to discriminate, and then attach to, both frogs and lizards under field and laboratory conditions (Lopez et al. 2005). The annelids (*Dero superterrenus*) preferentially attached to paper that had been treated by contact with frog skin, whereas ostracods (*Elpidium bromeliarum*) showed no preference for treated or untreated paper. The annelids showed a stronger preference to avoid attaching to lizards to which they were exposed, instead opting to attach to skin of frogs. Like annelids, the ostracods preferred to attach to frogs, which was suggested to simply be directly due to the relative difficulties in attaching to the lizard skin (Lopez et al. 2005). Evidently, this difference is related to the greater vulnerability of the annelids to desiccation as compared to the ostracods, which being crustaceans have some notable advantages over annelids

(presence of "hard valves" and a lack of a relatively delicate tegument foremost among them). The ostracods attach themselves to anything that enters the bromeliad water, but the annelids are reliant on chemical cues to trigger their phoretic behaviour. In addition, the ostracods can pass through the digestive tract of vertebrates alive, potentially meaning another avenue of phoretic transport is available, although such a method leaves the disembarkment up to chance and leaves the unfortunate ostracod possibly stranded in faeces, an unfavourable situation. In this particular scenario, the two phoronts live in the same habitat, have access to the same potential hosts and have the same issue in dispersing in a transient habitat; the annelid has developed a chemical sensitivity that favours hosts that share its issue with desiccation, whereas the ostracod is more flexible.

The egg parasitoid wasp, *Trichogramma brassicae,* can exploit a reproductive pheromone emitted by lepidopterans (Huigens et al. 2009). Adult females of *T. brassicae* are phoretic on butterflies of *Pieris brassicae* (Large Cabbage White), and use them as routes to parasitize the eggs laid by females. It is therefore no surprise that *T. brassicae* have developed a method that allows them to more efficiently focus their efforts. The wasps are sensitive to an antiaphrodisiac pheromone (benzyl cyanide) emitted by mated females of *P. brassicae.* These mated females are prime targets as the wasp can avoid choosing a host butterfly that does not transport the wasps to its target life stage, the butterfly's eggs. Instead, some of the inherent gamble in choosing a potential host that will be reproductively successful, is removed. Whereas adults of Trichogrammatidae are notably tiny, they are not wholly reliant on lepidopterans for dispersal because they do possess wings.

The first instar campodeiform larvae (known as triungulins) of parasitoid Strepsiptera engage in phoresy as a means of finding new hosts (Kathirithamby 2009). As a typical example, the triungulins of *Parasxenos lugubris* crawl onto *Ammophila* wasps while the wasps forage. The wasps carry the larvae back to the wasp nests where the triungulins disembark. The triungulins are sealed within individual cells and henceforth have unrestricted access to immature wasp larvae. In addition, internal phoresy has been reported in the Strepsiptera, having been noted by Linsley and McSwain (1957) (cited from Kathirithamby 2009). Strepsipteran larvae were ingested along with nectar by *Andrena complexa* and stored in the honey sac; as the bee provisioned its young with the expelled nectar, the strepsipteran larvae were regurgitated into the

cell. In this manner, the Strepsiptera achieve the same result with a slightly modified strategy. Reports of internal phoresy are somewhat uncommon, but since definitively determining if internal phoresy is occurring depends on either dissection or directly witnessing it, internal phoresy might be ubiquitous.

The triungulins of Strepsiptera possess a strategy similar to the triungulins of Meloidae (Coleoptera) despite belonging to different orders; they possess a similar body plan, in addition to being highly mobile and easily capable of crawling onto hosts. In a particularly bizarre scenario, the triungulins of *Meloe franciscanus* seem to be aggressive in their search for a host, adopting a strategy of making potential hosts search for the phoronts. These triungulins act cooperatively to lure male bees of *Habropoda pallida* into attempting copulation with a squirming ball of triungulin larvae that are thought to mimic both visual and chemical cues of a receptive female, in an effort to be transported to nests (Hafernik and Saul-Gershenz 2000). These triungulins are highly mobile, allowing them to make quick transitions from the male bees to the females. This switch to the other host sex allows for access to the bee nests, where the cells containing pollen, nectar provisions and the bee eggs are ready to be exploited.

1.1.3.2.4 Structures of attachment

Assuming that a phoront has overcome that important challenge of finding a host, the phoront must then overcome the obstacle of remaining attached.

An exceptionally early example of an attachment structure was found in a phoretic springtail and its mayfly host, preserved in 16 million year old amber (Penney et al. 2012). The ancient collembolan had its prehensile antennae attached to the base of the mayfly's wing. It should be noted that collembolan phoronts do still exist today, with the springtail *Cyphoderus similis* being phoretic on the fire ant *Solenopsis invicta* (Moser and Blomquist 2011).

Some other notable phoronts are the Braulidae or bee-lice, an atypical group of dipterans, which are primarily inquilines and have a simplified form for a life dependant on *Apis mellifera* as they feed on small amounts of wax and honey (Morse and Nowogrodzki 1990). The adults are heavily modified; they are small, lack wings and halteres, have reduced sensory organs, eyes and antennae, and have special comb-like claws to cling to the body hair of *A. mellifera*.

Mites have several notable methods of attachment as befits a group in which associations like phoresy are well represented. Sometimes the method of attachment is one of the primary aspects by which different "types" of phoresy are categorized, as proposed by Farish and Axtell (1971). They describe four basic types of phoresy in the Acari, which are generally restricted to specific mite families in which: a) the adult female is phoretic (Macrochelidae); b) the deutonymph stage is the phoront (Parasitidae); c) attachment occurs by means of an anal pedicel (Uropodidae); and d) a fourth type in Acaridae where the phoretic stage is the hypopus which is the heavily modified (morphologically simplified) deutonymph stage and resistant to desiccation, possessing many sucker-like discs for attachment.

These types of phoresy are distinguished on the basis of the different methods used by specific mite taxa to adhere to their host. In the Macrochelidae, adult female mites use chelicerae to hold onto setae or integument folds, whereas in Parasitidae, the ambulacral claws of the deutonymph are used. The anal pedicel in Uropodidae is formed by the extrusion of a substance from the mite anus that hardens when exposed to air and adheres the mite to its host (Faasch and Schaller 1966, cited from Farish and Axtell 1971). In some Acaridae, the hypopus has multiple sucker-like discs as part of the major morphological modifications that are only present in the deutonymph stage of life (Farish and Axtell 1971). This hypopus is heavily modified into a heteromorphic stage that is easily distinguished from other stages of the life cycle, having a reduced gnathosoma or mouth region (the hypopus does not feed) and possesses an extensive and complex arrangement of suckers on the ventral posterior surface which Binns (1982) variably called a posterior suctorial disk or suctorial ventrocaudal plate, and has been referred to elsewhere in the literature as a (ventral) caudal sucker plate (Houck 1994). There is a large variation between different types of phoresy because mites are especially common as phoronts (often with insects as their carrier host) and phoresy has developed independently in different mite groups (Binns 1982).

Phoretic mites usually have one stage that is specialized for dispersal and these stages can be classed into unspecialized homeomorphs, specialized homeomorphs and facultative heteromorphs (Houck and OConnor 1991): a) Unspecialized homeomorphs are similar to all other life stages and attachment is accomplished by use of structures already in use for other purposes (chelicerae and pretarsal claws); b) Specialized homeomorphs have some small

morphological changes that are used specifically for attachment (e.g., the specialized female in some Heterostigmata groups has enlarged fore legs and the deutonymphs of Uropodidae have their hyaline anal pedicel); and c) Facultative deutonymphs are optional stages that are highly specialized and appear markedly different in morphology compared to their taxon's particular body plan (the heteromorphs -referred to as hypopi- are characterized by a rudimentary gnathosoma, absence of a mouth, absence of a hollow gut, extensive scelrotization and a caudoventral attachment organ). Whereas the methods of attachment among mites can vary, there are certain features that are common in the phoretic stages of some unrelated mites, possibly examples of convergent evolution. The body shape is often oval or circular and somewhat flattened in anoetid and acarid hypopi, female scutacarids, certain members of tarsonemid and pyemotid mites, and uropodid deutonymphs (Binns 1982). Other features that occur among phoronts are long terminal leg setae on leg iv, strong leg i claws (characteristic in some tarsonemids and pyemotids), and a caudal suckerplate in acarid and anoetid hypopi. Some mites take advantage of host morphology, such as adult laelapids that ride in acarinaria present on carpenter bees (Houck and OConnor 1991).

1.1.3.3 Known halictid bee-mite associations

Phoresy in the Acari can be highly generalized (a mite utilizing multiple host species across different orders) to highly specific in both host choice and position on the host (mites having very specific positions on relatively few species) (Houck and OConnor 1991). Bees are widely utilized by mites as hosts partly due to their habits; with few exceptions, bees construct and provision nests with pollen and nectar, making them prime potential hosts for mites which can utilize the bees for transport, shelter, and food (Eickwort 1994). There are many mites which are heavily dependant on bees not just for phoresy, but also as inquilines which develop and can reproduce within the nest of the species used for transport. Mites are carried by adult bees into the nests, with those mites carried by adult females having access to the cells as the female constructs and provisions the nests. Different mites have different strategies to compensate for some of the notable differences between some of the eusocial bees (such as *Apis mellifera*) which practice progressive provisioning and those solitary and weakly social bees which practice mass provisioning, that is where a single cell is constructed, provisioned, oviposited within, and sealed by an adult female before moving on. Most mite lineages are restricted in their hosts and few

mite species occurring in social bees' nests share ancestry with those mites that primarily associate with solitary bees (Eickwort 1994). Those mites that associate with mass provisioners are often limited to a single cell, although some mites can move through the soil and invade the other sealed cells in a nest. Those bees that progressively provision and store their food separately from the brood must contend with mites that can infest their food reserves (Eickwort 1994).

It is believed that the relationship between mites and bees has evolved independently many times, with Eickwort (1994) hypothesizing that the symbiosis had at least 31 separate origins. Mites may have hosts spread across many bee taxa or be restricted to one or two subfamilies, and few mite species develop with only one host species when other species of the same genus are readily available in the same area. It is not particularly useful to use mites to clarify the phylogenetic relationships between bees due to a number of reasons. Mites that associate with solitary bees are often saprophytes or feed on pollen or honey; however, the few true parasites of bees are limited to highly social bees with most mites being commensal (Eickwort 1994).

Those mites that associate with bees are a diverse group, with representatives from Astigmatina (=Astigmata), Prostigmata, and Mesostigmata; of these, the Astigmatina were described by Eickwort (1994) as the most abundant mite associates of bees, many having developed a specialized phoretic deutonymph that is an integral part of a strategy that allows them to exploit transient, resource-rich habitats, like insect nests.

Owing to this study's investigation of a species of sweat bee, namely *Halictus rubicundus* Christ (Hymenoptera: Halictidae), previous studies of mites associated with bees of Halictidae are emphasized (Table 1.1). Indeed, some of the earliest reports of bee-mite relationships involve Halictidae, with the augochlorine bees *Oligochlora eickworti*† and *Oligochlora micheneri*† found in Dominican amber dated from Oligocene or Lower Miocene having phoretic mites present (Engel 1996). These mites belong to an unknown taxon, but were found in the weakly defined acarinarium and on the forewing of one female *O. eickworti*† and on the gena and ventral thorax of a female *O. micheneri*†. Among the soil-nesting bees, the Halictidae have some of the greatest diversity of mite associates (Eickwort 1994). There are representatives of Astigmatina, Prostigmata, and Mesostigmata among the mites associated with halictid bees, with the majority

of mites restricted to the subfamilies Nomiinae and Halictinae (Table 1.1). There is only one mite species, the not particularly discriminating *Imparipes apicola*, reported on a member of Rophitinae, considered to be the most primitive subfamily in Halictidae (Eickwort 1994). Many of the remaining mites are associated with the Augochlorini and Halictini tribes within the subfamily Halictinae. It must be noted that certain mites in Table 1.1 are not exclusive to Halictidae. As an example, *Imparipes apicola*, which appears multiple times within the table (i.e., as an associate of *Nomia melanderi* and *Evylaeus quebecensis*) is also reported as being an associate of other bees (Eickwort 1994). Many *Imparipes* species have been reported as bee associates, especially in ground-nesting bees, where the adult female mite can burrow through the soil and invade other nests; *Imparipes apicola* is singled out as one of the few mites that regularly develops in association with hosts belonging to more than one subfamily (Eickwort 1994). Should the bee larva die, the scutacarids can feed on the decaying brood and/or provisions; saprophagy is considered the primary method of gaining nutrients among the important mite associates of bees (Eickwort 1994). Other important mites in the table are members of the genera *Histiostoma* and *Anoetus*, belonging to the family Histiostomatidae within Astigmatina. The Histiostomatidae also includes the genus *Glyphanoetus*, but of the three genera, *Anoetus* is considered to be especially important, being defined as *only* containing obligatory associates of halictine bees (Mahunka 1974, cited from Eickwort 1994). *Anoetus* are the most commonly encountered mites in halictine nests worldwide, associating with multiple species of Halictidae, both solitary and social (Eickwort 1994). Along with members of the genus *Imparipes*, *Anoetus* spp. are noticeably common in Table 1.1.

Table 1.1 Mite associates reported from halictid bees with notes on bee social structure and mite life history. This table reports the currently accepted names for bee host and mite species. The publication's original names are given in brackets. This table includes data compiled from Eickwort (1994) and Fain et al. (1999). Mite taxonomy is based on Eickwort (1994).

Subfamily: Tribe	Bee species (Published name)	Social Structure	Mite associates (Published name)	Mite notes	Source
Halictinae: Augochlorini	*Augochlora cordiaefloris*		*Anoetus eickworti (Histiostoma eickworti)* **A**	Phoretic Hypopus Scavenger or saprophyte	Woodring 1973
	Augochlora nominata		*Anoetus eickworti (Histiostoma eickworti)* **A**	Phoretic Hypopus Scavenger or saprophyte	Woodring 1973
		Primitively social	*Anoetus* sp. **A**	Scavenger or saprophyte	Eickwort and Eickwort 1972
		Primitively social	*Parapygmephorus* sp. **P**	Scavenger or saprophyte	Eickwort and Eickwort 1972
	Augochlorella persimilis	Primitively social Form colonies	*Laelaspoides ordwayae* **M**	Phoretic Adult females Young mites eat pollen	Eickwort 1966
	Augochlorella aurata (*Augochlorella striata*)	Solitary Social (Packer 1990)	*Laelaspoides ordwayae* **M**	Phoretic Adult females Feed on pollen	Eickwort 1966
	Oligochlora eickworti†	Unknown	Unidentified		Engel 1996
	Oligochlora micheneri†	Unknown	Unidentified		Engel 1996
	Thectochlora alaris		*Thectochloracarus neotropicalis* **A**	Phoretic Hypopus Potentially mutualistic	Fain et al. 1999

Halictinae: Halictini	*Agapostemon angelicus*	Solitary Communal	*Parapygmephorus* sp. **P**	Scavenger or saprophyte	Eickwort 1981
Subfamily: Tribe	Bee species (Published name)	Social Structure	Mite associates (Published name)	Mite notes	Source
	Agapostemon nasutus	Parasocial? Quasisocial? (Eickwort and Eickwort 1972) Communal	*Parapygmephorus costaricanus* **P**	Scavenger or saprophyte	Rack and Eickwort 1979, as cited by Fain et al. 1999 Cross 1965, as cited by Fain et al. 1999
	Agapostemon sericeus	Solitary Communal Bivoltine and univoltine	*Sancassania* sp. **A**	Found feeding on moldy provisions	Eickwort 1981
	Agapostemon virescens		*Parapygmephorus halictinus* **P**	Scavenger or saprophyte	Cross 1965, Fain et al. 1999
	Halictus farinosus	Primitively or weakly eusocial colonies (Albert and Packer 2013)	*Trochometridium tribulatum* **P**	Scavenger or saprophyte	Cross 1965, as cited by Fain et al. 1999
	Halictus foanus	Solitary	*Imparipes paulyi* **P**	Phoretic Adult female Scavenger or saprophyte	Ebermann and Fain 2002
	Halictus geminatus		*Imparipes apidophilus* **P**	Phoretic Female Scavenger or saprophyte	Ebermann and Fain 2002
	Halictus graecus (*Halictus holtzi*)		*Anoetus szelenyi* **A**	Scavenger or saprophyte	Mahunka 1974, as cited by Fain et al. 1999
	Halictus jucundus	Solitary	*Imparipes paulyi* **P**	Phoretic Adult female	Ebermann and Fain 2002

				Scavenger or saprophyte	
Subfamily: Tribe	Bee species (Published name)	Social Structure	Mite associates (Published name)	Mite notes	Source
	Halictus ligatus	Social Form small colonies Continuously active Queens weakly dominant over workers (Packer 1985)	*Imparipes floridensis* **P**	Scavenger or saprophyte	Delfinado and Baker 1976b, as cited by Fain et al. 1999
	Halictus fulvocinctus		*Anoetus ligulotrichus* **A**	Scavenger or saprophyte	Mahunka 1974
	Halictus pollinosus (*Halictus pollinosus thevestensis*)	Solitary	*Imparipes paulyi* **P**	Phoretic Adult female Scavenger or saprophyte	Ebermann and Fain 2002
	Halictus rubicundus	Socially polymorphic (Albert and Packer 2013)	*Anoetus halictonida* (*Histiostoma halictonida*) **A**	Phoretic Hypopus Scavenger or saprophyte	Woodring 1973
	Halictus senilis	Solitary, weakly social (Miyanaga et al. 2006)	*Siteroptes cerealium* **P**		Cross 1965, as cited by Fain et al. 1999
	Halictus sexcinctus	Solitary, communal, eusocial, socially polymorphic (Richards et al. 2003)	*Anoetus halicticola* (*Histiostoma halicticola*) **A**	Phoretic Scavenger or saprophyte	Fain and Erteld 1998

Subfamily: Tribe	Bee species (Published name)	Social Structure	Mite associates (Published name)	Mite notes	Source
	Halictus tetrazonianellus		*Anoetus orientalis* **A**		Mahunka 1974, as cited by Fain et al. 1999
	Halictus sp.		*Anoetus tunisiensis* (*Anoetus tuniziensis* [sic]) **A**		Mahunka 1974
	Halictus sp.		*Halictacarus halicti* **A**		Mahunka 1974, as cited by Fain et al. 1999
	Halictus sp.		*Schulzea zachvatkini* **A**		Delfinado and Baker 1976
	Halictus sp.		*Schulzea* sp. **A**		OConnor 1988, as cited by Fain et al. 1999
	Lasioglossum atronitens	Solitary or Communal	*Raymentia walkeriana* **M**	Phoretic Adult female	Walter et al. 2002
	Lasioglossum eremaean	Solitary or Communal	*Trochometridium* sp. **P**	Unknown, suggested to be parasite of larvae in nest	Walter et al. 2002
	Lasioglossum lacthium	Solitary or Communal	*Raymentia eickwortiana* **M**	Phoretic Adult female	Walter et al. 2002
	Lasioglossum leucozonium	Solitary	*Sancassania* sp. **A**		Eickwort 1979
	Lasioglossum lineatulum (*Dialictus lineatulus*)	Primitively eusocial (Eickwort 1986)	*Anoetus* sp. **A**	Scavenger or saprophyte	Eickwort 1979
	Lasioglossum oceanicum		*Scutacarus eickworti* **P**		Delfinado and Baker 1976b,

	(*Dialictus nymphaearum*)				as cited by Fain et al. 1999
Subfamily: Tribe	Bee species (Published name)	Social Structure	Mite associates (Published name)	Mite notes	Source
	Lasioglossum quadrinotatum		*Anoetus alicola* **A**	Scavenger or saprophyte	Woodring 1973
	Lasioglossum quebecense (*Evylaeus quebecensis*)		*Imparipes apicola* **P**	Fungivore	Delfinado and Baker 1976b, as cited by Fain et al. 1999
	Lasioglossum titusi		*Imparipes vulgaris* **P**	Phoretic Fungivore	Ebermann and Hall 2003
	Lasioglossum umbripenne (*Dialictus umbripennis*)	Primitively social (Eickwort and Eickwort 1971)	*Anoetus debilis* **A**		Woodring 1973
	Lasioglossum versatum (*Dialictus rohweri*)		*Imparipes ithacensis* **P**		Delfinado and Baker 1976b, as cited by Fain et al. 1999
	Lasioglossum zephyrum (*Dialictus zephyrus*)		*Anoetus vexarus* **A**		Woodring 1973
	Lasioglossum sp.		*Imparipes apicola* **P**	Fungivore	Delfinado and Baker 1976b, as cited by Fain et al. 1999
	Lasioglossum sp. (*Dialictus*)		*Imparipes apicola* **P**	Fungivore	Delfinado and Baker 1976b, as cited by Fain et al. 1999
	Lasioglossum sp. (*Dialictus*)		*Imparipes neotropicus* **P**	Fungivore	Delfinado and Baker 1976b, as cited by Fain et al. 1999

	Lasioglossum sp. (*Evylaeus*)		*Imparipes mexicanus* **P**	Fungivore	Delfinado and Baker 1976b, as cited by Fain et al. 1999
Subfamily: Tribe	Bee species (Published name)	Social Structure	Mite associates (Published name)	Mite notes	Source
	Megalopta sp.		*Anoetus halictonida* (*Histiostoma halictonida*) **A**	Phoretic	Engel and Fain 2003
Halictinae: Sphecodini	*Sphecodes arvensiformis*		*Trochometridium tribulatum* **P**		Cross 1965, as cited by Fain et al. 1999
Nomiinae	*Lipotriches tomentifera*		*Neocypholaelaps* sp. **M**	Phoretic Flower mite (feeds on pollen and nectar)	Walter et al. 2002
	Nomia melanderi	Solitary	*Sancassania boharti* (*Caloglyphus boharti)* **A**	Necrophage Phoretic Hypopus	Cross and Bohart 1969 Cross and Bohart 1991
			Imparipes apicola **P**	Fungivore Phoretic as Adult female	Cross and Bohart 1991
			Glyphanoetus nomiensis **A**	Phoretic Hypopus	Cross and Bohart 1969
			Trochometridium tribulatum **P**	Phoretic Adult female	Cross and Bohart 1969
			Imparipes apicola (*Imparipes americanus*) **P**	Phoretic adult female Fungivore (potentially mutualistic)	Cross and Bohart 1969 Eickwort 1979

			"Nanacarus" nominis **A**		Woodring 1966, as cited by Fain et al. 1999
			Trochometridium tribulatum **P**	Scavenger or saprophyte?	Cross 1965, as cited by Fain et al. 1999
	Nomia nortoni		*Glyphanoetus nomiensis* **A**	Scavenger or saprophyte	Cross 1968,as cited by Fain et al. 1999
	Nomia sp.		*Nasutiscutacarus ampliatus* **P**	Scavenger or saprophyte	Beer and Cross 1960, as cited by Fain et al. 1999
	Nomia strigata	Solitary (Gonzálvez and Rodríguez-Gironés 2013)	*Nasutiscutacarus anthrenae* **P**	Scavenger or saprophyte	Beer and Cross 1960, as cited by Fain et al. 1999
Rophitinae	*Dufourea novaeangliae*		*Imparipes apicola* **P**	Phoretic Adult female	Eickwort et al. 1986

1. † indicates an extinct species.
2. **A** indicates a member of the cohort Astigmatina.
3. **P** indicates a member of suborder Prostigmata.
4. **M** indicates a member of order Mesostigmata.

The foundation for this thesis was a project supported by a NSERC Undergraduate Student Research Award during May-August 2013, with the purposes of: a) surveying the University of Saskatchewan collection of pinned bees (excluding members of Apidae) and specifically cataloguing which of those bees had mites and other associates, and b) collecting foraging bees from around the city of Saskatoon and noting mite associates. Among the adult bees collected, *Halictus rubicundus* was of interest due to its relatively large numbers of distinctive, phoretic mites. Woodring (1973) reported that *H. rubicundus* has the mite, *Histiostoma halictonida*, as a known associate (Table 1.1). Thus, the preliminary study of

summer 2013 was extended with the purpose of specifically investigating the relationship between *H. rubicundus* and its mite associate.

1.1.4 Objectives

The three goals of this research project were to investigate

a) the nesting biology of the sweat bee, *Halictus rubicundus*, at two urban sites on the University of Saskatchewan campus in the bee's northwestern range, with emphasis on subterranean nest architecture, the pollen types provisioned for the bee larvae, and the phenology of the life cycle stages of this bee species (Chapter 2);
b) the identity, morphology, and differential distribution of the single mite species commonly associated with this sweat bee's immature and adult stages according to sex, plus some notes about mites associated with a small number of additional halictid bee species encountered at the study sites (Chapter 3); and
c) some interactions between the common mite species and adults of *H. rubicundus*, such as potential for mite transfer to other individual hosts, and the theoretical impact of phoretic mite loads on bee flight or balance (Chapters 3 and 4).

CHAPTER 2 – LIFE HISTORY OF THE SWEAT BEE, *HALICTUS RUBICUNDUS* [CHRIST] (HYMENOPTERA: HALICTIDAE)

2.1 Introduction

2.1.1. Bee phylogeny and the family Halictidae

Like wasps, bees are Aculeata – members of the insect order Hymenoptera which possess stings - and are thought to have arisen from the sphecoid wasps (Spheciformes) (Michener 2007). Specifically, it was proposed that bees arose from within Crabonidae (Debevec et al. 2012). Bees are aculeatans which, over evolutionary time, switched from provisioning their young with prey to gathering floral resources, namely nectar and pollen, but also floral oils, resins and, in the genus *Trigona,* carrion (Packer et al. 2007). Bees are distinguishable from wasps based on the presence of branched hairs and longer length of the hind basitarsi (Michener 2007). Worldwide, it is estimated there are 20,000 spp. of bees, 4% of which are believed to be represented within Canada (Sheffield et al. 2014, Guidotti 2016). Despite its north temperate climate, bee diversity in Canada is extensive, with 797 confirmed species and a slightly higher estimate of 800-825 species, representing six of the nine families that occur worldwide (Packer et al. 2007, Sheffield et al. 2014, Raine 2016, Suzuki 2016).

The family Halictidae, also known as sweat bees, is present worldwide and tends to numerically dominate most other bees in temperate areas (Michener 2007). In eastern Canada specifically, 109 of the 409 species of bees (26.6% of the area's diversity) are accounted for by this single family (Packer et al. 2007). There is disagreement, however, in the taxonomic treatment of members of this family. According to Michener (2007), the Halictidae comprises four subfamilies: Rophitinae, Nomiinae, Nomioidinae, and Halictinae, with the latter being divided into two tribes, Augochlorini and Halictini, with Halictini including the subtribe Sphecodina. However, Danforth et al. (2008) instead recognize five tribes within Halictinae: Augochlorini, Thrinchostomini, Caenohalictini, Sphecodini, and Halictini; this taxonomic designation was applied in Table 1.1.

2.1.2 Designation of solitary and social behaviour among bees

Bees are highly variable socially, ranging from the highly and truly social (i.e., eusocial) to the isolated, solitary species. Highly eusocial bees, namely the honey bees and stingless honey bees, belong to only two tribes in Apidae, the Apini and Meliponini (Michener 2007). These social insects have a reproductive caste, the queen, unable to survive by herself and attended by her many daughters, which themselves cannot reproduce. These are some of the only bees that practise progressive provisioning, the continual feeding of the larval stage until larvae pupate. On the other side of the social scale are the solitary bees, whose life style is extremely common worldwide. A solitary female bee will make her own nest and provide food for her brood, lacking assistance from other bees and usually dying before her offspring matures (Michener 2007). A solitary bee practices mass provisioning, gathering a large amount of pollen and nectar into a mass that is stored in the cell before ovipositing an egg. This sealed provision is all the individual larva will receive from its mother, and it will fuel transition from larva to adult with this set amount of food. Between these solitary and highly eusocial levels, there are several degrees of sociality with terms to describe the variability: subsocial, colonial, eusocial, primitively eusocial, communal, semisocial, parasocial, and quasisocial. The differences between these occasionally overlapping terms are outlined in Table 2.1.

Table 2.1 Terms describing different social arrangements in bees, adapted from Michener (2007).

Social type	Solitary	Subsocial	Colonial	Eusocial	Primitively eusocial
	One female founds a nest.	Similar to a solitary bee; single female constructs and provisions the nest, but differs in how she provides for her offspring.	Two or more adult females in a single nest.	There is a division of labour among adult females of two generations. Considered an adequate term for most bees.	Nests founded by a single female which performs all necessary functions (construction, foraging, provisioning, etc.) until the emergence of daughters.
	The female provides food, but is not a progressive	Feeds and cares for offspring (as opposed to	Females either workers (forage,	Some of the females (mothers) lay the eggs, the	Division of labor between the foundress queen and

	provisioner and does not provide ongoing care.	storing food for them). Lacks assistance from other bees. No castes and no division of labour.	guard, care for offspring) or a queen (lays the eggs). Division of labour present.	daughters act as foragers.	daughter-workers. Queens and workers morphologically similar, but behaviourally distinct.
Examples, if available			Some social halictines.	Applies to permanent honey bee colonies, as well as temporary halictine colonies, and bumblebee colonies.	Bumblebees (*Bombus*), Some Halictinae.
Social type	Communal	Semisocial	Parasocial	Quasisocial	Highly eusocial
	Two or more females in the same nest.	Multiple females of the same generation, often sisters.	Umbrella term for a colony where the social structure is unknown.	Multiple females inhabit a nest. Cooperation, but no definite queen.	Presence of castes and division of labour. Different castes highly dependant on each other.
	Females construct and provision their own cells. Lack of division of labour. Lack of castes.	Division of labour present. One female lays the eggs and the others forage.	Impossible to recognize relationships. Could be communal, semisocial or quasisocial.	Cooperation in building and provisioning cells. Multiple females lay eggs in the cooperatively constructed cells.	There is a single egg-laying female attended to by many females (generally her daughters). Progressive provisioning and brood care.
Examples	Communal living is facultative and almost definitely	Considered to be very close to primitively eusocial arrangements	Placeholder term.	Very uncommon. May be exhibited by colonies at	Honey bees, stingless bees.

	underreported. Other individuals in the same species and even population may be solitary.	(the queen dies and one of her daughters replaces her).		times. Thought possibly be an unstable or transition state.	

Various terms are utilized to define traits of social bee behaviour. For example, aggregations are groupings of nests in a limited area; the individual nests may be solitary or somewhat social. The foundress is the female that founds a nest, and is almost always mated, but unmated females that attempt to found nests have been reported (Yanega 1989). The term gyne is used for both potential and functional queens, but is most often used for the females that will or might become queens, as opposed to established queens (Michener 2007). Soucy (2002) uses the term gyne for any potential nest foundress and non-gyne for a female that remains in an already established nest, and may or may not have offspring, but does not have the ability to undergo diapause. Other useful terms are workers, which are non-gynes that maintain the nest by foraging and guarding, and replacement queens, non-gynes that become the dominant egg layer when the colony's previous queen disappears (Soucy 2002). Bees, like most hymenopterans, have a haplodiploid system of sex determination, in which the ploidy of the eggs determines the sex of the resulting bee, unfertilized eggs developing into males, and fertilized eggs developing into females (Michener 2007). The mated female stores the sperm and can control the sex of the egg by releasing or withholding the stored sperm (Michener 2007).

The degree to which a bee species is social or solitary can be variable, with some bees displaying social flexibility based on several factors (see *Halictus rubicundus*, described as socially polymorphic, in Table 1.1). Soucy (2002) reported *Halictus rubicundus* as facultatively social, being solitary in areas where the climate is cool, social in warmer climates, and displaying a combination of social and solitary behaviour in marginal environments. Not only does *H. rubicundus* show variable nesting behaviour across its range, but occasionally within the same population, with social populations undergoing a solitary phase early in the season as foundress females establish nests (Yanega 1988, Soucy 2002).

For instance, populations of *H. rubicundus* in New York are among the most studied of the species. Generally, *H. rubicundus* is referred to as eusocial, a more general term indicating division of labour between females, and to be more specific, is classed as primitively eusocial (terms outlined in Table 2.1) (Cane 2015). The New York population described by Yanega (1990) rarely had greater than 300 individuals active at a time. Mated females overwinter away from the nest aggregation site and can remain in diapause for 8 to 11 months (Yanega 1990). These females are philopatric and will return to excavate individually, constructing new nests very close to where they were raised (often within 50 cm of their natal nest) (Yanega 1990). Foundresses initiating nests occasionally become disoriented and this may lead to abandoning their first excavation and beginning another, but very rarely does one female usurp and displace another foundress (Yanega 1990). A single female returns to the nesting site around April or May and begins producing a few cells over a month-long period (Yanega 1990). In the second generation the mean productivity is 1.5 offspring produced per worker (Yanega 1989). Broods with multiple mothers are rare, as are instances of a female displacing the original nest excavator (Yanega 1990). Within the nest aggregation, individual nests are made by constructing a tunnel which, by the end of the first brood production, will terminate in a cluster of cells; the nest will later be enlarged to make room for the second brood (Soucy 2002). It is uncommon for workers to enter or reside in non-natal nests, but approximately a third of males will return to a nest, either natal or non-natal, and patrol the area for females (Yanega 1990).

At first, each foundress must provision her nest by herself (Soucy 2002). Typical of mass provisioning, each constructed cell is supplied with a pollen ball upon which a single egg is laid, and finally sealed before construction begins on the next one (Soucy 2002). The brood cells were reported at the depth of 3-10 cm, and buffered from daily temperature fluctuations (Yanega 1993). The female foundress begins provisioning in early May and continues through mid-June, and it takes a little less than 2 weeks from the end of provisioning the final cell of the first brood, until the first adults emerge from her nest, around the middle or end of June (Yanega 1989). The first brood is weakly protogynous, and the workers began provisioning in late June through early July, with a few very late-initiated colonies provisioning until July 30 (Yanega 1989). Brood emergence continues into mid-August, but the second brood was male-biased and contained fewer total bees than the first brood (Yanega 1988, 1989). Two female castes, gynes and non-gynes, are produced, differentiating early in their adult life: the gynes leave the population to

overwinter, while non-gynes remain and differentiate into workers and replacement queens (Yanega 1989). The caste differentiation appears to be behavioural, and there is a lack of morphological and physiological differences (Yanega 1988). The gynes are larger on average compared to non-gynes, although the relationship is not considered to be causal, with females that emerge later tending to be larger (Yanega 1989). The reason for differentiation between gynes and non-gynes is thought to be linked to whether the females mate promptly soon after emerging (becoming gynes that leave to undergo diapause) or if they do not (Yanega 1989). The first brood comprises mostly females that act as workers and most do not mate promptly (Yanega 1988). The females that emerge later (still in the first brood) are increasingly likely to mate as more males emerge. Those females that do mate leave the nesting site to hibernate and become potential nest foundresses the following year; the workers and replacement queens do not undergo diapause, but many will mate and contribute to the current season's population (Yanega 1988). When workers emerge, the queen remains within the nest, laying eggs while her daughters provision until the middle of July (Yanega 1988, Yanega 1989). In the second brood, sexes are more equally represented, and the females do not act as workers (Yanega 1988, Yanega 1989, Yanega 1993). Instead, the females mate and leave to hibernate like the females from the first brood which did not become workers (Yanega 1988). It is due to the presence of both first generation and second generation individuals during emergence of adult bees of the second brood that *H. rubicundus* has been described as partially bivoltine (Yanega 1990).

In comparison, *H. rubicundus* was reported as having a solitary life cycle in the high-altitude, subalpine region of the Colorado Rocky Mountains (Eickwort et al. 1996). This particular population had an average of 6.5 offspring per foundress and only approximately half of the foundresses survived to offspring maturity (Eickwort et al. 1996). There was an almost equal investment in male and female offspring, with males emerging first (i.e., broods were protandrous) (Eickwort et al. 1996). The authors note that the male-biased single brood produced by this population more closely resembled the second brood described by Yanega (1989), rather than the worker-dominated first brood (Eickwort et al. 1996). Outside of this location, *H. rubicundus* is reported as social (Eickwort et al. 1996). The species has annual colonies, and two separate broods at minimum during the season; as described above from New York, U.S.A., the first brood is female biased with only some males and most of the females from the first brood become workers, whereas the second brood has males and females that mate (Eickwort et al.

1996). The females of the second brood enter diapause after mating and will be the ones to initiate colonies in the next year (Eickwort et al. 1996). *Halictus rubicundus* has certain life cycle modifications that allow for solitary behaviour at high-altitudes (Eickwort et al. 1996). The flight season during which the broods are produced is too short at high altitudes to allow for this system of having two broods, which means that the sweat bees which inhabit such areas must be solitary (either facultative or having evolved solitary behaviour) or otherwise adapted (Eickwort et al. 1996). The social structure of *H. rubicundus* is thought to be sensitive to environmental influence by means of abiotic factors altering sex-ratios (Yanega 1993). There is a correlation between ambient temperature during the provisioning phase and the sex-ratio of the brood that results, with warmer temperatures favouring an increase in males (Yanega 1993). Warmer temperatures resulting in more males are expected to result in the differentiating of a greater proportion of gynes (the period of differentiation into the gyne or non-gyne caste is believed to occur when the female is an adult, and is dependant on how soon the female mates) (Yanega 1993). More males mating with the newly emerged females results in more gynes, which will leave and enter diapause, and fewer workers, leading to smaller colonies (Yanega 1993).

2.1.3 Foraging behaviour and pollination biology

The pollinating activities of a bee depend on its plant preferences and foraging habits. Some bees are oligolectic and will restrict pollen gathering to one or a few closely related species of plants. However, this term refers specifically to pollen specialists, not nectar specialists, because even oligolectic bees may visit a wide range of plant species to gather nectar. Polylectic bees gather pollen from more than a few plant species, and hence are much less discerning in their choice of floral resources. Polylecty is fairly common in Canada, with many bee species acting as generalist pollinators (Packer et al. 2007).

The discussion of floral resources and social complexity is loosely related to the distinction of short-tongued and long-tongued bees. Long-tongued bees, including the social honey bees, are considered to be the more derived bees, whereas the short-tongued bees are considered more ancestral (Eickwort 1994). Short-tongued bees are restricted in their access to certain types of flowers because they lack the morphological adaptations of their advanced cousins. This distinction is only partially true: there is enough variability among tongue length and flower morphology (i.e., corolla length) that some long-tongued bees still cannot successfully

gather pollen and nectar from certain flowers and some short-tongued bees are able to outperform. The andrenid bee, *Andrena lonicerae*, belongs to a family grouped within the short-tongued bees. Due to coevolution with the honeysuckle *Lonicera gracilipes*, the oligolectic *A. lonicerae* is the flower visitor which manages to collect the majority of nectar produced by *L. gracilipes* (Shimizu et al. 2014). Other flower visitors gather pollen from *L. gracilipes*, but *A. lonicerae* has an elongated tongue allowing it to access the nectar within elongate, narrow corollas of *L. gracilipes*.

The halictine species, *Halictus rubicundus*, described by Michener (2007) as belonging to the subgenus *Protohalictus* [Pesenko], is holarctic, and has been reported from all over North America: from Alaska, U.S.A. and Nova Scotia, Canada, in the north, and to Texas and Florida, U.S.A., in the south. On emerging from diapause in spring, mated *H. rubicundus* females excavate nests and forage for pollen and nectar, especially from plant species belonging to Asteraceae and Rosaceae (Soucy 2002). In this study, the nesting biology of the sweat bee, *H. rubicundus*, was investigated at two urban sites on the University of Saskatchewan campus. This study provides information regarding the life history of the widespread *H. rubicundus* from its northwestern range in North America, an area from which there is no work present in the literature. The aspects that were given special attention were the subterranean nest architecture, the pollen types provisioned to the bee larvae, and the phenology of the life cycle stages of this bee species.

2.2 Materials and Methods

A preliminary project (May-August, 2013) that preceded this graduate research project on *Halictus rubicundus*, had two concurrent parts: a) surveying the University of Saskatchewan collection of pinned bees for any mites and other associates, and b) capturing foraging bees from within the city limits of Saskatoon and surveying these individuals for any associated arthropods. The survey of the university's collection excluded bees belonging to Apidae, instead focusing on all other bees within the collection. Similarly, the collection of live bees from Saskatoon was originally unrestricted, but later dedicated to Andrenidae, Colletidae, Halictidae, Megachilidae, and those apids that were not *Apis mellifera* or *Bombus* spp. Capture was achieved by netting bees as they foraged, with the flowers being collected and placed in 70% ethanol for later dissection under a microscope to determine if there was any overlap between associates on bees

and those on flowers (to indicate whether any of the associates on bees might use the flowers as a way to transfer between hosts). Specimens of the university's pinned collection and my summer 2013 collection were viewed under a dissecting microscope and marked (either with slips of paper or red tape added to the pins) for further reference such as determining the type of associate and their distribution on the bee's body. Most of the pinned bees in the university collection only had mites, with one exception of a beetle larva (likely Meloidae)(see Appendix A.1), and these mites were easily removed due to their desiccation, by nudging with a pin. Mites were also the most commonly encountered associate on the newly captured bees, being readily apparent on Halictidae and Megachilidae, whereas strepsipterans were only found on andrenids (Appendix A.2).

2.2.1 Nesting sites of *Halictus rubicundus*

The preliminary study initiated during summer 2013 continued as this graduate project during 2014 and 2015 with a much narrower focus on the sweat bee, *Halictus rubicundus*, which was both plentiful and had impressive numbers of a very distinctive mite. Early work involved finding two nesting sites on the University of Saskatchewan campus, having similar characteristics. Of the two, one was far superior in numbers of nests and bees; site 1 is located in an obviously man-made rock garden (Fig 2.1a) on the east side of the College of Education Building where Education Road meets Education Crescent (52°08'06.9"N 106°38'09.0"W). The area is approximately J-shaped, about 8m long, 3.5m wide at the shortest point and 7m at its widest. The rocks are fairly large, most ranging from 0.2-0.4m wide, and deeply sunk into the dirt, which is mostly clay in some places. There is sparse vegetation (Fig 2.1b) that increases as the season progresses, mostly on the "hook" of the J-shape, which is the site's most northern part and is well shaded by the building, flora and detritus from previous years. This same area is less utilized by nesting females of *H. rubicundus* than the more barren area between the rocks (Fig 2.1c). Nests are densely packed in certain areas, sometimes with two nest entrances, approximately 3-4 mm in diameter (Fig 2.1d), only 1 cm apart on the surface. Occasionally the bees nest along the margins of the large rocks and follow the rock edge down for a short distance before nest tunnels extend further into the soil without an obvious guide.

Figure 2.1. Site 1 located outside east entrance of the College of Education Building, University of Saskatchewan, late spring 2015. a) View of the site, from the south, showing large rocks of variable size sunken in ground. b) Rocks are separated from each other; the sparse plants do not obscure the soil until late summer when adult bee foraging has decreased. Ruler is 31 cm long. c) Holes demarcating nest entrances occasionally were densely aggregated in soil between rocks. d) Nest entrance (approximately 3-4 mm diameter) next to ruler.


a


b


c


60 70 80 90 100 110 120 130 140 150
d

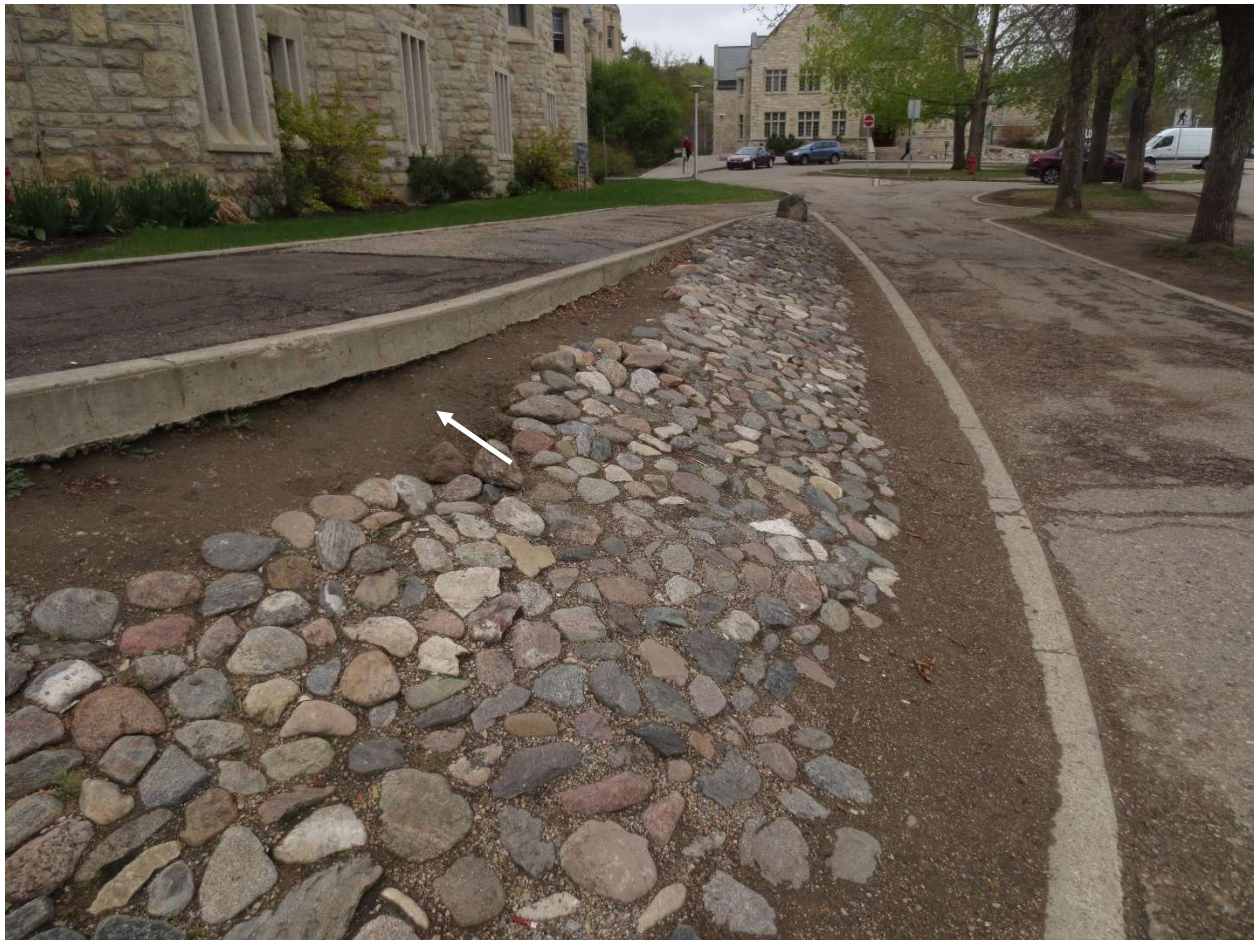


Fig 2.2. Site 2, located immediately south of Qu'Appelle Hall, University of Saskatchewan. Site consists of a sloped and rocky area with little space between rocks and almost no plant life. The majority of nests identified were located at the margin of the concrete pathway indicated by the arrow.

The second site (Fig 2.2) is located in front of Qu'Appelle Hall and between that student dormitory and the road entering the bus terminal circuit (52°07'49.6"N 106°38'10.3"W). The area was wedge shaped, about 20 m long and slightly exceeding 2m at its widest point, on a south facing slope that is bordered by an overhanging lip of concrete, under which the soil has partially eroded and slopes down to a pathway. The area was dominated by medium sized (0.1 m) rocks deeply set into hard clay dirt. There was little to no vegetation and the nest entrances of *H. rubicundus* were usually found under the overhanging concrete lip or between the rocks.

2.2.2 Nest architecture and excavation of nest contents

Nest excavation occurred typically once per week from May to August in each field season (2014, 2015). Nests at the site 2 (Qu'Appelle Hall) were only excavated during 2014 because of low numbers of nests and bees. Site 1 (Education Building) was richer and hence became the main focus of the 2015 field season. Excavation was done via the common method of Plaster of Paris molding. This method involves forcing freshly prepared Plaster of Paris, sometimes using a plastic squeeze bottle, into subterranean bee nests where it can dry and hence outline the nest architecture without filling the nest's sealed cells (Eickwort 1969). Whereas other methods are available for outlining the boundaries of a nest (e.g., using visible powders that can be blown into the nest or substituting aluminum for Plaster of Paris to achieve a permanent and faithful representation of the nest; see Tschinkel 2010), the utilization of Plaster of Paris was judged to be the best option for this study. Multiple nests were excavated simultaneously to avoid damaging or impacting adjacent nests. Adult bees in the nest were easily captured and the liquid Plaster of Paris was usually cleaned off by the bees grooming themselves. Excavation took place early in the morning, typically 8 or 8:30 am in the spring months, but excavating had to be begin earlier (7:30am) later in the year as the site warmed up faster, to capture the bees before they left their nests to forage. Plaster of Paris (DAP brand) (approximately 3.5 cups water to 6 tablespoons of plaster) was poured into the nest entrance using a funnel or, to allow for finer control, a straw (0.5 cm diameter and 20.3 cm long). The straw method was the more commonly used and involved using a thumb or finger to trap liquid plaster in the straw via vacuum. After insertion into the nest, the plaster was allowed to dry partially, generally after waiting approximately 15 min. The plaster rarely dried completely and could not be left unattended as the bees would easily dig themselves out of the nest. For excavation, the nest was dug using hand tools (i.e., trowel,

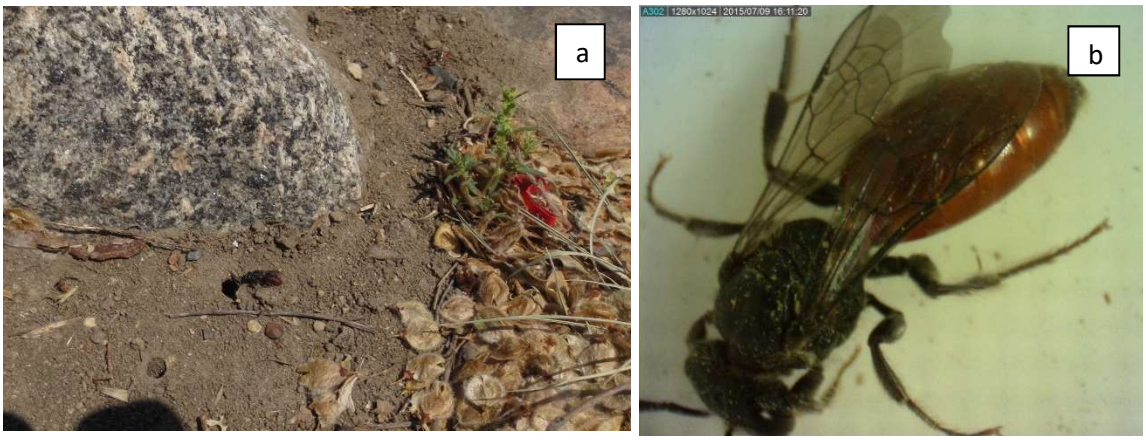


Fig 2.3. Detection of *Sphecodes* (Halictidae) at site 1. a) Female *Sphecodes* inspecting nest entrance of *Halictus rubicundus* July, 2015. b) Image of female *Sphecodes* taken with Dinocapture in the lab.

paintbrush, paint chipper) and photographs were taken to record nest layout as dirt was removed. Bees of all life stages, pollen provisions, and several cells from the nest were put into separate, labelled glass vials and frozen for future reference. However, the majority of observations were made at the time of excavation.

2.2.3 Other bees nesting and reproducing at the sites

Whereas the majority of bees observed at site 1 were *H. rubicundus*, other bees were also found there. Although no immature specimens were located, five female *Sphecodes* (Halictidae) were captured (Fig 2.3b) at site 1 during the 2015 season. *Sphecodes* were observed scouting the area, landing, moving from nest entrance to nest entrance (Fig 2.3a), and occasionally entering. The genus *Sphecodes* is known for its common moniker "cuckoo bee" and its method of reproduction involving cleptoparasitism. Female *Sphecodes* never construct nests or provision their own young, instead taking advantage of other bees to provide these necessities.

There was one instance of a nest of *Lasioglossum* (Halictidae) at site 1. No confrontations were recorded between this bee and *H. rubicundus*. Also noted at the same site were two nests of an unknown megachilid. Only larvae enclosed in leaf cases were recovered. It is possible that the megachilids utilized previously constructed nests of *H. rubicundus* given the proximity, but the exact relationship between these two bees is unknown. In 2014, megachilids were also nesting at site 2, although many of the nests were destroyed by Richardson ground squirrels (*Urocitellus richardsonii*). The squirrels had unearthed the leaf cases and consumed the contents relatively soon after construction (i.e., the destroyed leaf cases were still green). The squirrels dug shallowly (approximately 4-6cm) and it is unlikely they consumed many if any *H. rubicundus* due to the difference in nest depth.

2.2.4 Light and scanning electron microscopy of bees

The bees frozen in their labelled vials were removed and examined under a dissecting microscope. Some images were taken in the lab using a Dinocapture camera (AnMo Electronics Corporation) placed on the eyepiece of the dissecting microscope, connected to a laptop computer. Several of the bees which had arthropod associates were placed on double-sided tape on aluminum stubs before coating using a Gold Sputter Coater (Edwards S140B) and then imaged with the Phenom G2 desktop scanning electron microscope (SEM), located in the

Microscopy Resources Facility of the Department of Biology. In certain instances, the bees were dismembered before mounting on the stubs to allow for greater visibility of certain body parts.

2.2.5 Mass of adult bees

Eight adult female and male bees, selected randomly to represent multiple dates of capture throughout the 2015 field season, were weighed on a microscale (Dr. Diane Knight's lab in the Department of Soil Science, College of Agriculture and Bioresources).

2.2.6 Microscopy and determination of average weight of pollen provisions

Of the 43 total pollen provisions recovered during nest excavations, 18 particular pollen provisions were selected to be dried and weighed. Selection of the pollen provisions was restricted to those that were discrete pollen masses from the 2015 field season, and were not contaminated with dirt or fungus. The pollen provisions were originally allowed to air dry, but then were dried overnight in an oven at 60 ºC. After weighing, these pollen provisions were rehydrated, broken apart with the head of an insect pin and allowed to dry in weigh boats before undergoing preparation for SEM microscopy (see section 2.2.4) to improve the taxonomic identification of pollen collected by the foraging *H. rubicundus*.

Some of the pollen provisions - those provisions unsuitable (see above) for drying and weighing - were used to quickly screen the taxonomic range of pollen grains present using light microscopy, before it was determined that SEM might be more useful in determining the numerous species present. Small samples of the provisions were removed with a pin, placed in a dish and stained with either aqueous toluidine blue or methylene blue. The stains were diluted, one drop of stain in 6-7 ml of water, and the pollen grains allowed to sit for 6 min. The pollen grains were then transferred to a glass slide under a coverslip and examined under a compound microscope. Images were taken (Dinocapture apparatus of section 2.2.4) at varying magnification.

2.3 Results

2.3.1 Nest architecture

The overall shape of each subterranean nest of *H. rubicundus* is generally a single tunnel (Fig 2.4a,b), approximately 0.4 cm in diameter. Nests excavated after late June, 2015 were

deemed to be close to completion, extending downward at least 30 cm, but not exceeding 50 cm. Only 2 (2.6%) of the 78 nests excavated had multiple entrances. These dual openings were immediately apparent due to the much quicker flow of molten plaster down the nest, with the entrance not being filled acting as an expedient way for air to escape. Nests early in the year were vertical, but shallower, and the brood cells were within 10 cm of the surface (Fig 2.5). Although nest entrances were typically a minimum of 1 cm apart, sometimes brood cells of adjacent nests were located nearby (Fig 2.5). As the season continued, the cells could be found deeper, with the progression toward maturity of the cell inhabitants generally reflecting this trend (i.e., pupae that were more heavily pigmented were found closer to the surface).

2.3.2 Immature stages of *Halictus rubicundus* and nest phenology

Each cell has one translucent, curved, rice grain-shaped egg resting in a dimple on a packed pollen provision (Fig 2.6a). Each egg is about 3 mm long (Fig 2.6c).

The larvae are pale, approximately 1 cm long in their later stages, and C-shaped. They are found curled around their respective pollen provisions (Fig 2.6b, d). After consuming their pollen, the larvae defecate, leaving an orange-brownish mass on the cell wall furthest from the tunnel (Fig 2.7a).

Interestingly, pupae can be sexed by many of the same characteristics as adults (i.e., number of antennal segments, number of abdominal segments, general body shape). An estimate of pupal age can be made by the degree of sclerotization. Very young pupae are entirely pale (Fig 2.7b), whereas slightly more mature pupae have the beginnings of pigmentation in their compound eyes. Advanced pupae have sclerotization in the exoskeleton throughout the body (Fig 2.6e), and pharate individuals are basically adults, but still covered by an exuvium. Each pupa is generally oriented with its head toward the tunnel (Fig 2.6e) and away from the site of the first larval defecation (Fig 2.7b). This alignment seems to be decided fairly early on, with late stage larvae already oriented as such. One adult bee was found in its cell with its head oriented away from the tunnel, but was dead and heavily infested with fungus, having died after maturing. With 78 total nests excavated and the 192 individuals collected in which their anterior end could be distinguished from their posterior, this misalignment within a brood cell was uncommon (0.52%).

Fig 2.4 Nests of *Halictus rubicundus* at site 1. a). Image of nests that are not obscured by plaster of Paris. The nests follow the curvature of the removed rock (not pictured). b) Partially excavated nest (May 13, 2015) showing pollen provisions (arrows) within the first 5 cm of the surface. Several tunnels filled with plaster of Paris (stars) are also evident here.

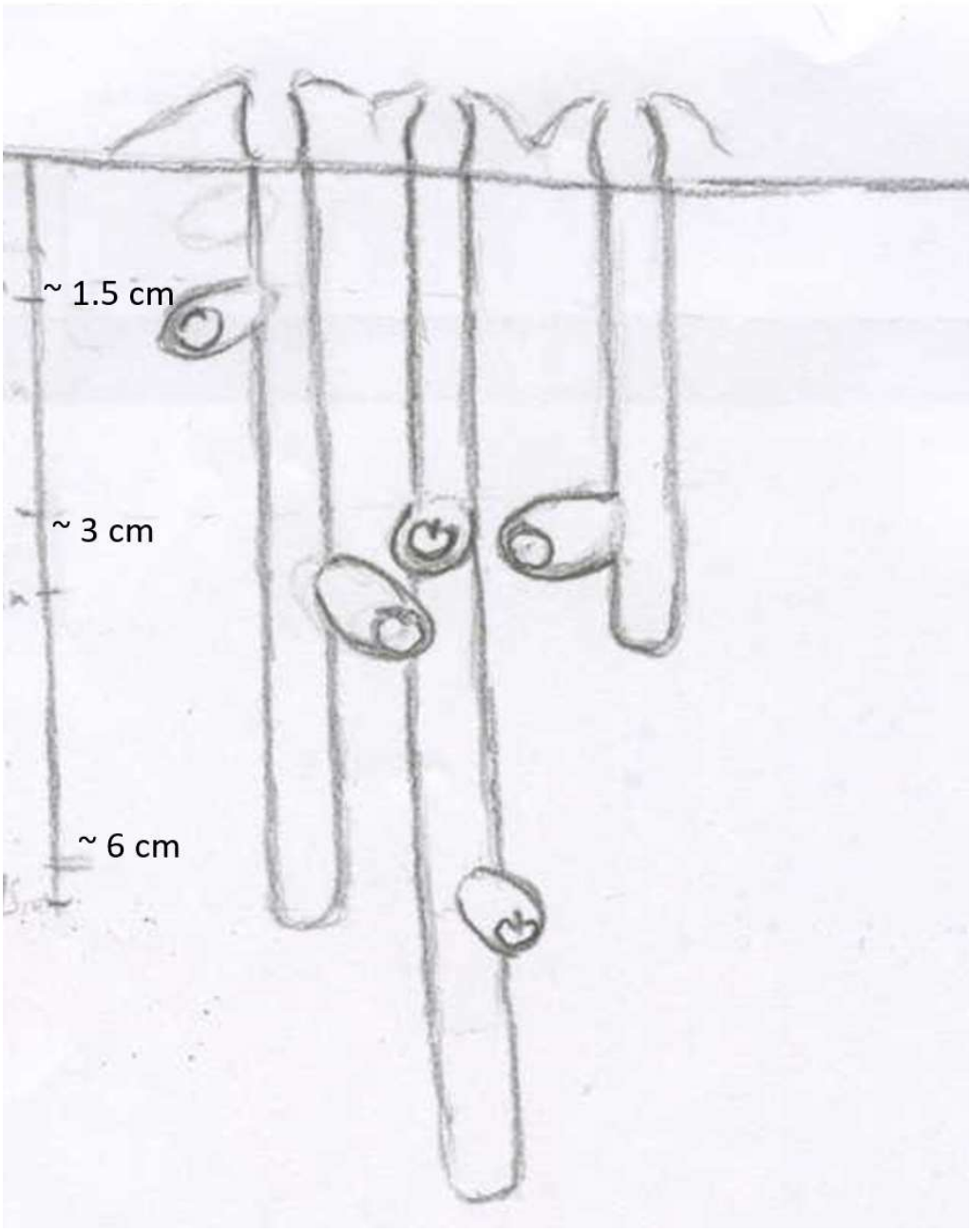


Fig 2.5. Drawing of a cluster of subterranean nests of *Halictus rubicundus* depicting three of the seven excavated on May 13, 2015. Nests were not especially deep or complex. All of the five cells shown contain what would be considered complete pollen provisions, but one (far right) is missing an egg. To the left are the approximate depths. Note that whereas the tunnels are often a minimum distance apart, some of the cells of adjacent tunnels are constructed closely together.

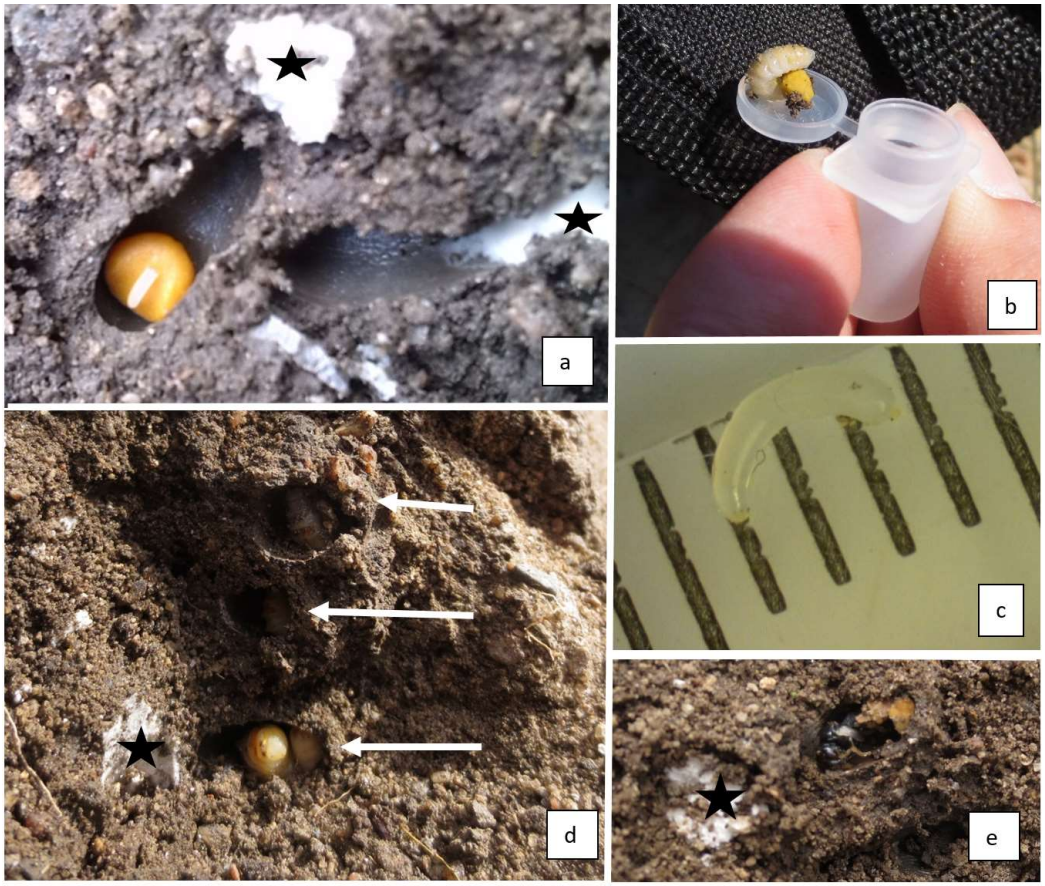


Fig 2.6. Assortment of the immature stages of *Halictus rubicundus* at site 1. a) Two adjacent nests with opened cells visible, one cell with intact bee egg on sculpted pollen provision. Cells are smooth, slightly wet looking, and sealed off, preventing them from being filled with plaster of Paris. b) A late stage larva curled around its pollen provision. c) A close-up of the bee egg removed from its associated pollen provision and placed against a ruler showing 1 mm gradations. d) A partially excavated nest showing three cells, each containing a later stage larva, situated in a column. e) A mature pupa with head oriented towards the plaster-filled tunnel (star).

Fig 2.7. Immatures of *Halictus rubicundus* that have yet to be removed from their cells within excavated nests; the upper portion of each cell is flipped over nearby. a) Exposed larva in cell with pollen provision having been consumed entirely. The larva's anterior end is directed towards the plaster-filled tunnel. Note that the top part of the cell (circle) shows evidence of the first defecation. b) An early stage pupa, lacking sclerotization, lying in its original orientation within its brood cell, the top portion of the cell (circle) having its basal end laden with fecal material and fungal growth.

As summer 2015 progressed, the proportion of nest inhabitants changed (Fig 2.8). The graphs are limited by which nests were excavated on a particular day, with some life stages and pollen provisions possibly undetected at certain times of the season due to sampling intervals. Generally, a nest was selected for excavation only on the basis that it was known to be inhabited by *H. rubicundus*, with adjacent nests (inhabited or not) also being excavated as they would also unavoidably be destroyed during the unearthing process. This way of sampling may have introduced a bias against the numbers of uninhabited nests indicated in the lowermost graph of Fig 2.8. Of the 78 nests excavated from May 1-August 14, 2015, 13 (16.7%) lacked nest contents and may therefore have been uninhabited.

Early in the year the only inhabitants were single adult females in partially constructed nests which lacked any immature or even unfinished pollen provisions (e.g. May 1 N1) (Fig 2.8). Within 6 days, pollen provisions and even the occasional early-stage larva were found (e.g. May 7 N1). Pollen provisions (all pollen provisions, even those unfinished) were present throughout the field season, although there were far fewer later in the summer (Fig 2.8). The final pollen provisions were found on August 3 (e.g., N1 and N3) and were accompanied by adult males, but not always by adult females. Around late May and early June, there was a noticeable peak in the number of larvae collected. The larvae recorded in Fig 2.8 are not separated into different categories, instars or sexes, because the characters used to distinguish adult or pupal females from males are not present and there was some uncertainty whether an early-stage larva could be distinguished from a late-stage larva. At no point during summer 2015 did the frequency of larvae reach this level again, although larvae were still collected late into the field season. After this peak of larvae, there was a much more gradual and dispersed peak of pupae, as the early-season larvae evidently reached maturity and underwent metamorphosis throughout June.

As the season continued, some nests apparently began to show signs of adult mortality, certain nests being host to pupae, but not to adult females. It is, however, possible that these females were not dead, only absent, despite excavation early in the day. Other nests were inhabited only by adult males (e.g., July 5 N6) or only by immature pupae and adult males (e.g., August 3 N3). The obvious assumptions are that one or more female bees constructed a nest, died, and the nest's only inhabitants represented opportunistic males that have taken to living in a non-natal nest, the original female inhabitant's progeny, or some combination. There are several

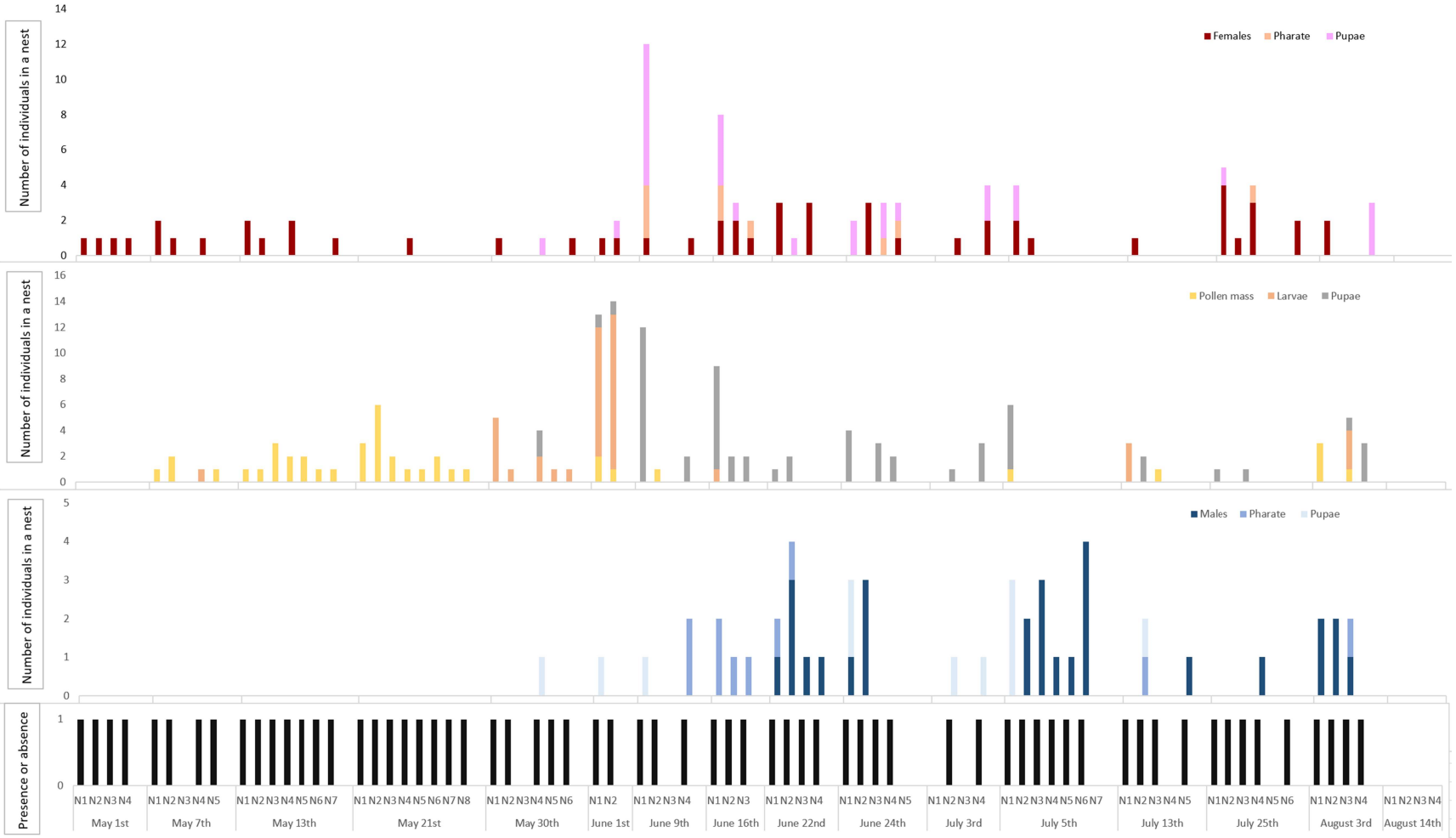


Fig 2.8. Seasonal progression of phenology of *Halictus rubicundus* depicting changes in bee life stages recorded among nest inhabitants during May 1-August 14, 2015. The topmost graph shows all stages of female bees. The second graph shows the occurrence of pollen provisions, plus all of the larvae, and pupae. The third graph illustrates the male stages. The lowermost graph indicates presence (1) or absence (0) of any nest contents. Labels along the X axis denote dates of nest excavations and numbers of nests excavated on a particular day, with the designations being reused (ie. N1 is the first nest unearthed on a given date). Note that the vertical (Y) axes are at different

Fig 2.9. Examples of the putative nest guarding behaviour of *Halictus rubicundus*. Behaviour exhibited by both females (red arrow, far right 2.9b) and males (blue arrows, 2.9a, b); each male is identifiable by its yellow clypeus. In Fig. 2.9a (white circle), note an example at site 1 of an ant, *Formica podzolica,* which occasionally antagonized adults of *H. rubicundus* and attempted to carry off *H. rubicundus* larvae which had been experimentally excavated from a nest.

2.3.3 Notes on behaviour of adult *Halictus rubicundus*

Bees were generally unaggressive and did not sting the observer, except for a period in early May in which there were a large number of females active. Nests were often found being obstructed or guarded, in which a bee remained at the mouth of the nest, blocking the entrance with its body. Usually this behaviour was observed in females (Fig 2.9b), but once males became active they could also be seen engaging in this activity (Fig 2.9a, b). Whether what the males are doing is truly guarding or represents a similar behaviour performed for a different purpose (i.e., watching for receptive females, or controlling its body temperature), is unknown. It was deemed unlikely that copulation occurs underground due to the mating posture involved. Some rivalry was noted to occur between bees and the adjacent ant colony of *Formica podzolica* (Fig 2.9a) that overlapped in places with the halictid nest aggregation, especially along the margins, at site 1.

Grooming was sometimes observed when bees landed and rested on the numerous rocks at site 1. The bees used their hind legs to clean their wings, balancing on fore and middle legs while lifting their hind legs up to apply pressure. The legs (evidently the tibia and tarsal segments) scraped along the wings in a motion traveling down the length of the abdomen and that kept the wings firmly pressed against the abdomen. Due to their orientation, the undersides of the wings are slightly more difficult, although not impossible, to groom. Dorsal areas were also difficult to reach due to anatomical limitations. Being accessible at the body's anterior end, the head, eyes, and antennae were more easily cleaned with the use of the forelegs.

Males were occasionally observed flying at and latching onto females in flight, causing both to tumble to the ground. Males could also be observed resting on rocks during the warmer days, and hiding under rocks and vegetative debris when it rained. The final individuals of *H. rubicundus* to be observed in 2015 at site 1 were two adult males resting on rocks, as opposed to occupying an excavated nest, on September 16.

2.3.4 Mass of adult bees

A few individual adults of both sexes were selected and cleaned (i.e., any plaster and mites were removed), then weighed (Table 2.2). All of the bees (n=8) were collected from nests during excavations and left unpinned. The females lacked pollen on the legs as they had not just

returned from a foraging trip and therefore had not recently consumed nectar. The bees were partially coated in plaster of Paris during the excavations, but the bees themselves are fairly adept at grooming. Remaining plaster of Paris was removed with pins and tweezers. Whether there is a difference between the females, for example, whether foundresses are heavier than workers is not known, mainly due to there being a lack of noticeable morphological differences between castes. A large range in body size of adult bees was detected within each sex, with females and males varying 2.3-fold and 1.7-fold, respectively. The average mass of female bees exceeded that of males (Table 2.2).

Table 2.2. Mass of adult bees of *Halictus rubicundus* sampled from site 1 in summer 2015 and cleaned of their mite loads before weighing.

	Female				Male			
Collection date	May 13	June 22	June 22	July 5	June 22	July 5	July 5	July 5
Mass (mg)	19.045	12.168	12.895	8.271	10.596	6.672	10.540	6.310
Mean ± S.D.	13.09 ± 4.46				8.53 ± 2.36			

2.3.5 Mass of pollen provisions from excavated nests of *Halictus rubicundus*

Pollen provisions recovered from nests of *H. rubicundus* during excavations at site 1 in May, 2015, averaged 36.1 ± 9.7 mg [n=18, representing May 7 (n=3), May 13 (n=5), and May 21 (n=10)].

As outlined in Section 2.2.6, these 18 pollen provisions represent random samples that were intact. They were selected to be dried and weighed for the specific purpose of determining average weight of the provisions throughout the month of May. These pollen provisions are distinguished from those pollen provisions listed previously, which were weighed, but then also destructively subsampled in order to examine pollen types comprising the provisions. The pollen provisions ranged from 17.0 to 57.8 mg. Part of this disparity may reflect the degree of completion before recovery from excavated nests. The 8 pollen samples selected did not appear to have undergone any decrease due to larval consumption. The average pollen mass for May, 2015 was 38.6 g, when including all corrected dry weights. Throughout these collection dates,

there was no overall pattern evident in mass of pollen load, but appears to decrease slightly (51 mg to <41 mg).

2.3.6 Identification of pollen grains comprising larval provisions of *Halictus rubicundus*

Pollen grains from eight destructively sampled pollen provisions were photographed and then assigned to a type, based primarily on the grain's morphology and when it appeared in the season (Table 2.3). Pollen grains were originally photographed using light microscopy (Fig 2.12), but afterward the majority of images (Fig 2.10, 2.11, 2.13-2.17) were acquired by scanning electron microscopy.

Table 2.3. Proportion (%) of the most abundant pollen types identified in the selected samples of pollen provisions taken from six nests of *Halictus rubicundus* excavated from site 1 on May 13, May 21, and June 1, 2015. The code in the left hand column indicates the date of the excavation (Month Day) and nest (N#).

Pollen sample	Mass of pollen provision (mg)	Type i	Type ii	Type iii	Type iv	Type v	Type vi	Type vii	Type viii	Type ix	Type x	Type xi	Type xii
Ps3 513 N1	50.3	97.1	2.1	0.5	0.2								
Ps4 513 N5	51.8	94.6			0.5								
Ps5 513 N5	51.9	96.2				1.9							
Ps6 513 N4	53.1	98.6											
Ps1 521 N2	50.2						1.9	60.6	14.7	3.9			
Ps2 521 N2	16.9							68.2	24.6	0.8	5.9		
Ps7 61 N1	40.8					4.1	30.9					22.7	7.2
Ps8 61 N1	N/A											98.3	

Often, pollen provisions were highly homogenous in nature (Fig 2.10), particularly those recovered on the earlier excavation date of May 13, 2015 (Table 2.3) wherein the most abundant pollen type exceeded 94% (Table 2.3). However, especially from nests unearthed late spring and early summer, pollen provisions were noticeably heterogenous (Fig 2.11), wherein the most plentiful type of pollen grain (Type VI in Ps7; Type VII in Ps1 and Ps2) only accounted for an estimated 30.9-68.2% of the entire provision (Table 2.3).

Of the eight provisions investigated microscopically in order to quantify the four most frequent types of pollen per provision, Type I (Fig 2.10, 2.17a) predominated (94.6-98.6%) throughout all nests excavated on May 13, 2015 (Table 2.3). Interestingly, provisions Ps4 and Ps5 recovered from both brood cells of the same nest N5 were very similar (Table 2.3). The minor pollen Types II-V (Fig 2.19, 2.20) never exceeded 2.1% and were not recorded again after May 13, 2015 (Table 2.3), suggesting those taxa may represent early, ephemeral floral sources, at least compared to Type I pollen. Pollen type I appears to have been a pollen type favoured by multiple females (Fig 2.13-2.16). The presence of other pollen grains indicates that this was not the only pollen available (Fig 2.13b), but the relative rarity of those types indicates that the pollen was not preferred. The bees may have been visiting the flowers (e.g., for nectar), but the females foraging in May did not seem to be actively collecting other types. Most of the pollen collected appears to be the same type. Despite some differences in length and width, the grains show many of the same characteristics such as number of furrows and exine patterning (Fig 2.15).

Although a highly homogenous provision from nest N1 (98.3% of Type XI pollen; Fig 2.21) was recovered on June 1 (Table 2.3), provisions from May 21-June 1, 2015, typically were heterogenous (Table 2.3). Interestingly, Type XI ranked second (22.7%) from the other brood cell of the same nest N1 on June 1, behind Type VI pollen (30.9%; Fig 2.19) which occurred in two provisions on May 21-June 1, 2015 (Table 2.3). Type VII (Fig 2.20) prevailed in the two provisions of May 21 (Table 2.3). Pollen types V (Fig 2.20), X (Fig 2.14), and XII (Fig 2.21) were less abundant (Table 2.3). Provisions collected later in the year showed more variation in pollen types, such as a greater number of Asteraceae (Fig 2.25 and 2.26), and may reflect a greater selection of floral resources available as early summer arrived. A large number of miscellaneous pollen grains (Fig 2.17) occurred in minor proportions per provision, typically less than 1.9% of a provision's pollen. Whereas certain pollen types were numerically better represented, they may be less important in the diet due to the large difference in pollen grain size.

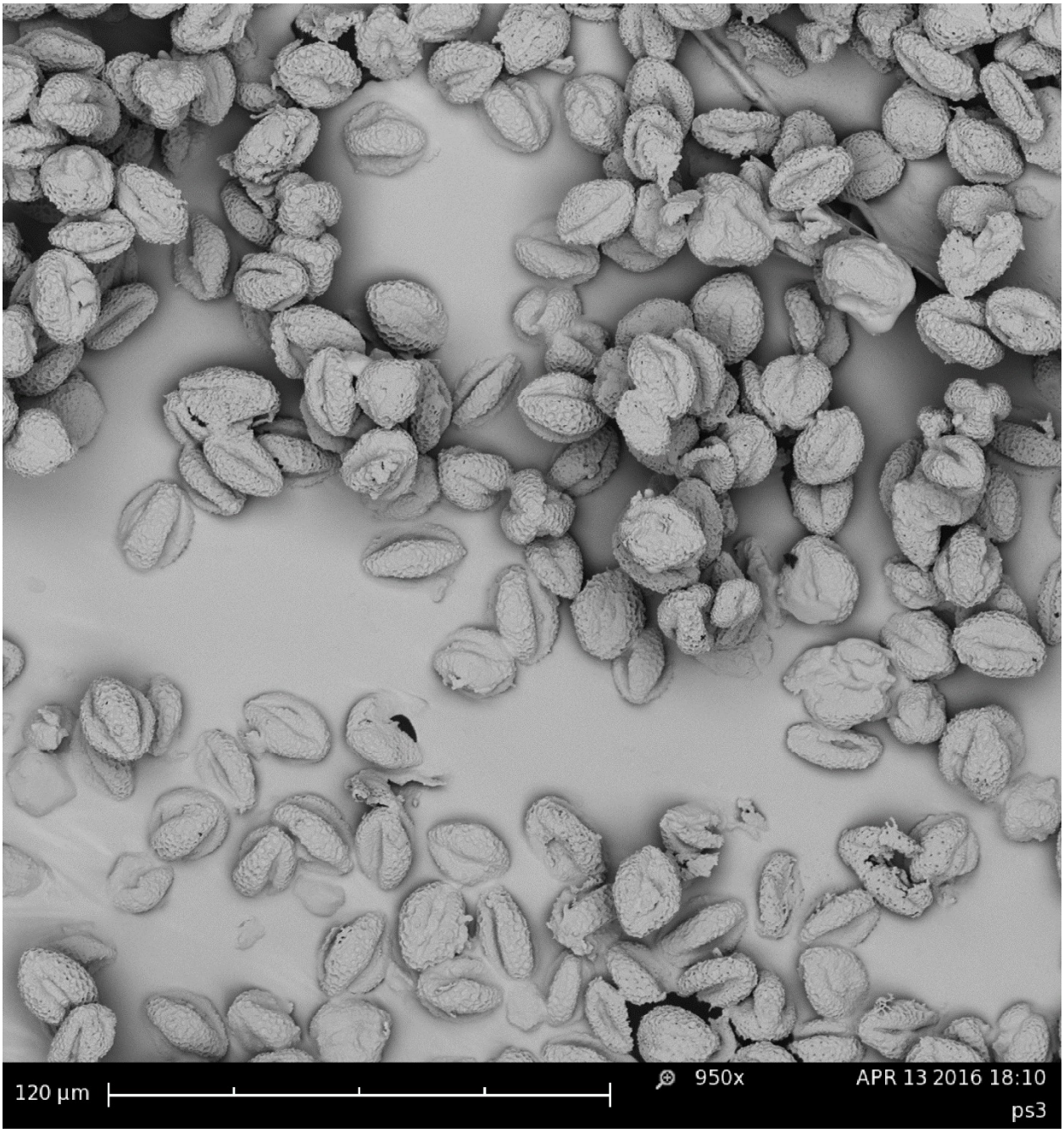


Fig 2.10. Pollen subsample from provision Ps3 of nest N1 of *Halictus* rubicundus collected at site 1 on May 13, 2015. Note the clearly homogeneous nature of this pollen provision, in which almost every pollen grain is identifiable as Type I.

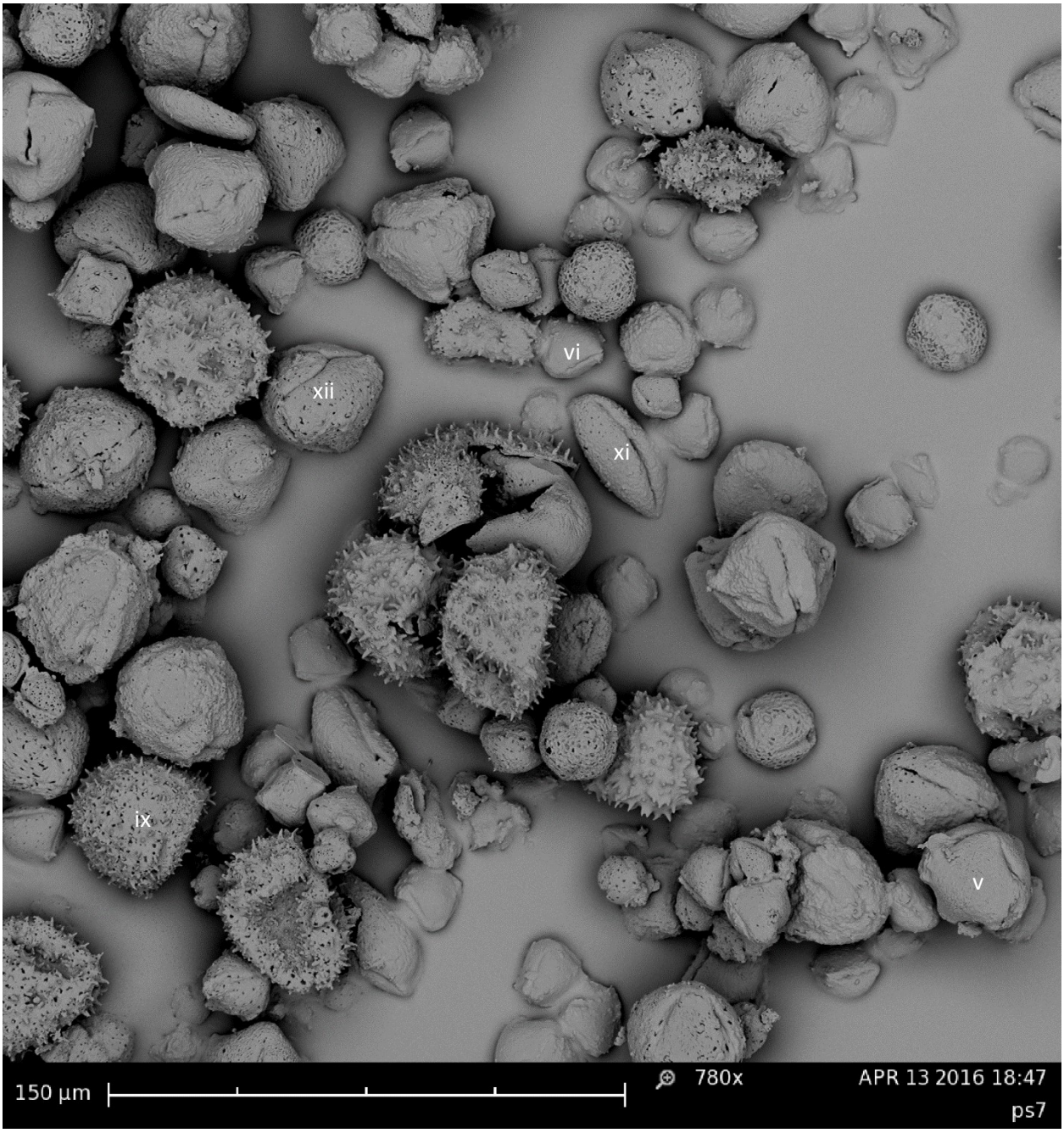


Fig 2.11. Pollen subsample from provision Ps7 of nest N1 of *Halictus rubicundus* collected at site 1 on June 1, 2015. Note the highly heterogeneous nature of this pollen provision, depicting greater than five different pollen types. The large spiky, sculptured pollen grains, evidently of the Asteraceae, became much more apparent later in the season, along with several other pollen types that are absent or poorly represented in other samples.

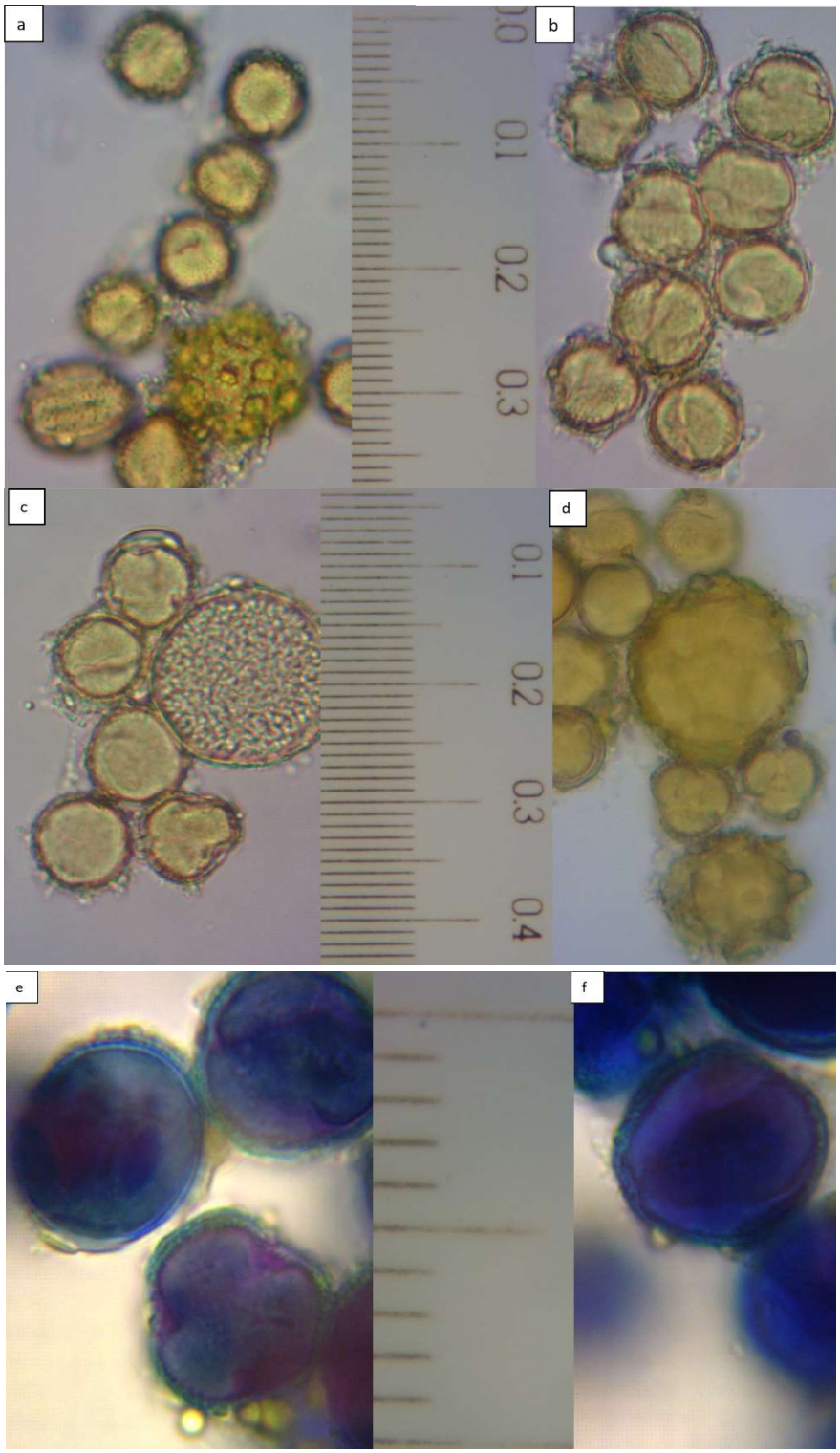


Fig 2.12. Stained and unstained pollen grains from provisions recovered in late May and early June from excavated nests of *Halictus rubicundus* at site 1. a-d) Unstained subsamples showing what appears to be a mix of Asteraceae as well as possible Type I, or XI. e-f) Stained subsamples showing possible Type I or XI. Scale bar with increments of 0.01 mm.

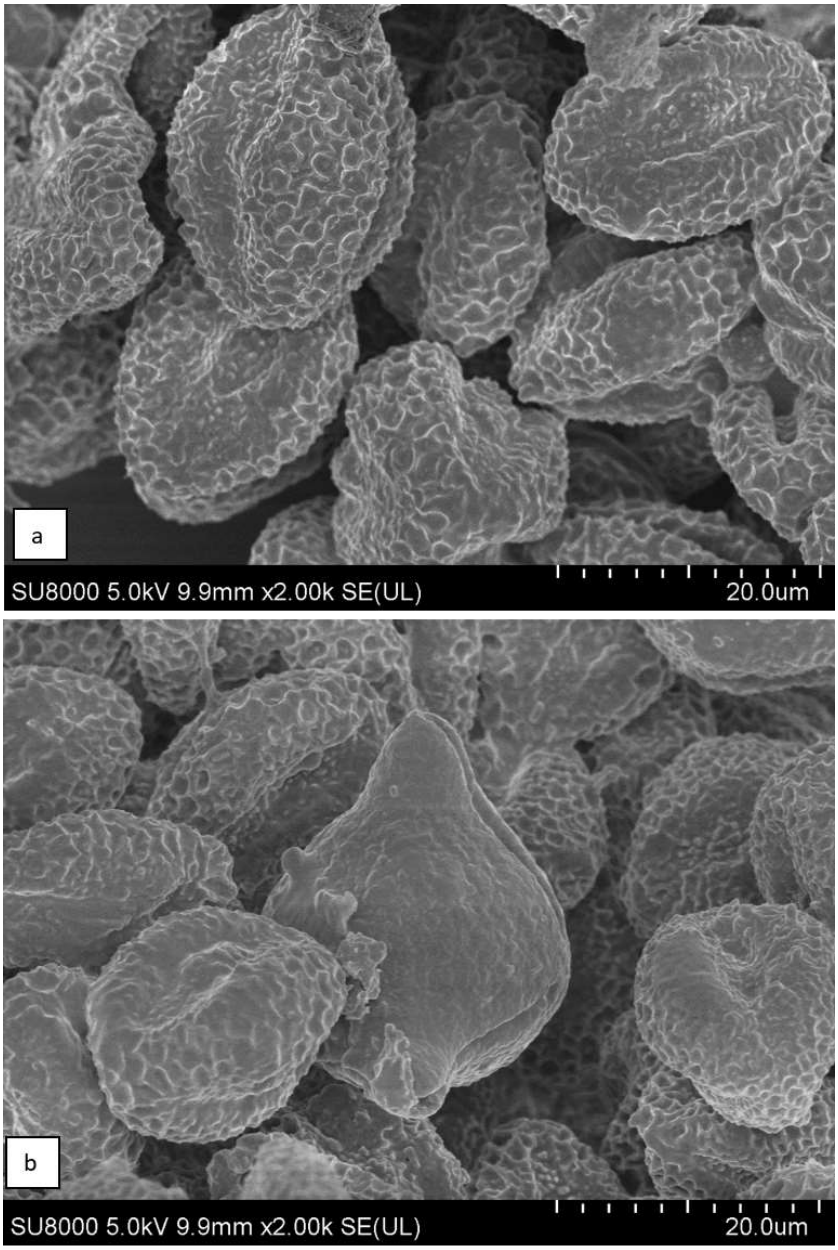


Fig 2.13. Pollen from Ps3 showing predominantly Type I and what may be Type IV. Sample was generally homogenous as shown in a), with tri-furrowed and highly sculptured pollen accounting for the majority. All pollen grains are thought to be the same type despite differences in shape. Pollen shows similar overall structure (three furrows) and exine patterning, despite variabilities in length and width. Besides this primary pollen type, other noticeably different pollen grains were uncommon b), but not absent.

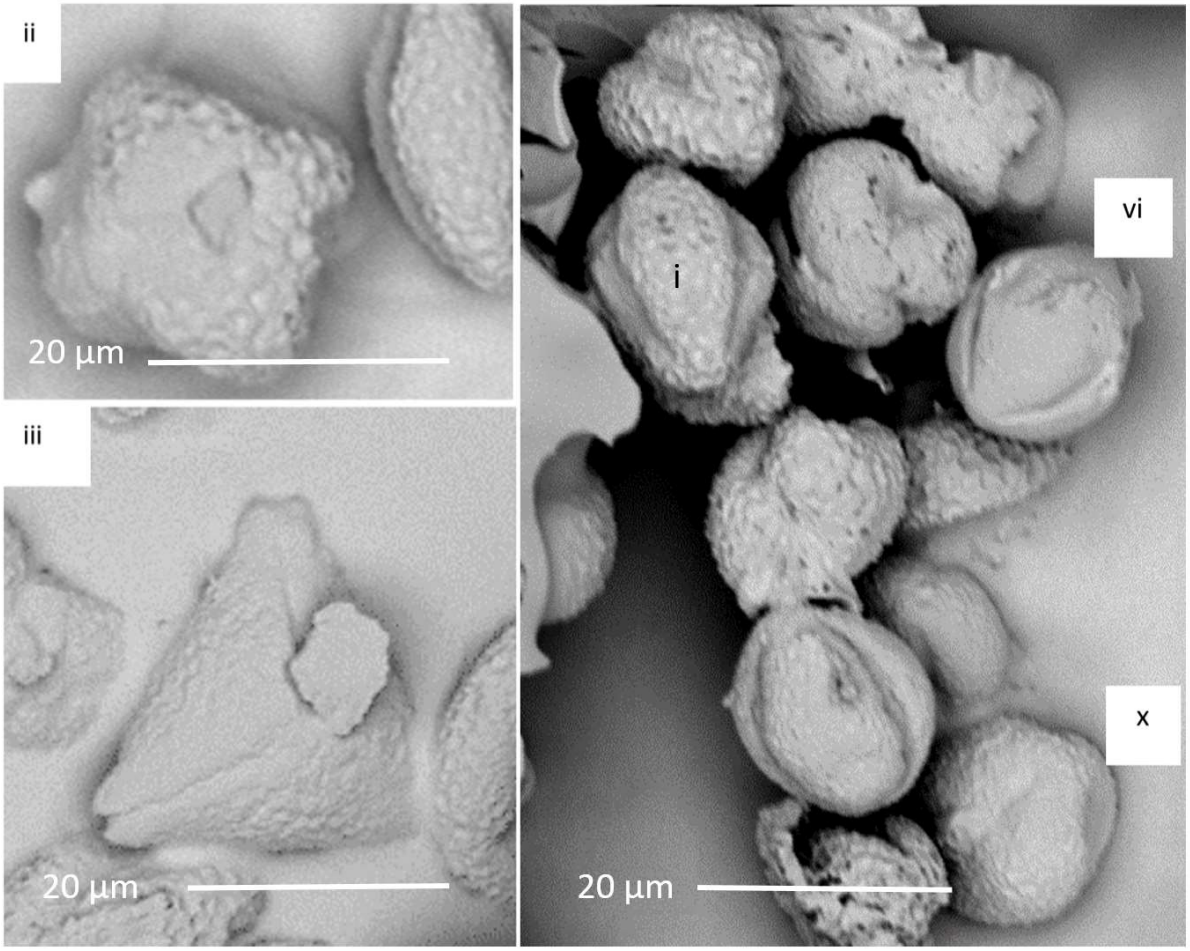


Fig 2.14. Pollen types II (four furrows, reticulate exine patterning), III (triangular, flattened pollen, slightly bumpy texture), VI (three furrows, comparatively smooth), and X (three furrows, rougher texturing than type vi, smoother than type i) identified from pollen provisions of *Halictus rubicundus* recovered from excavated nests at site 1 in May, 2015.

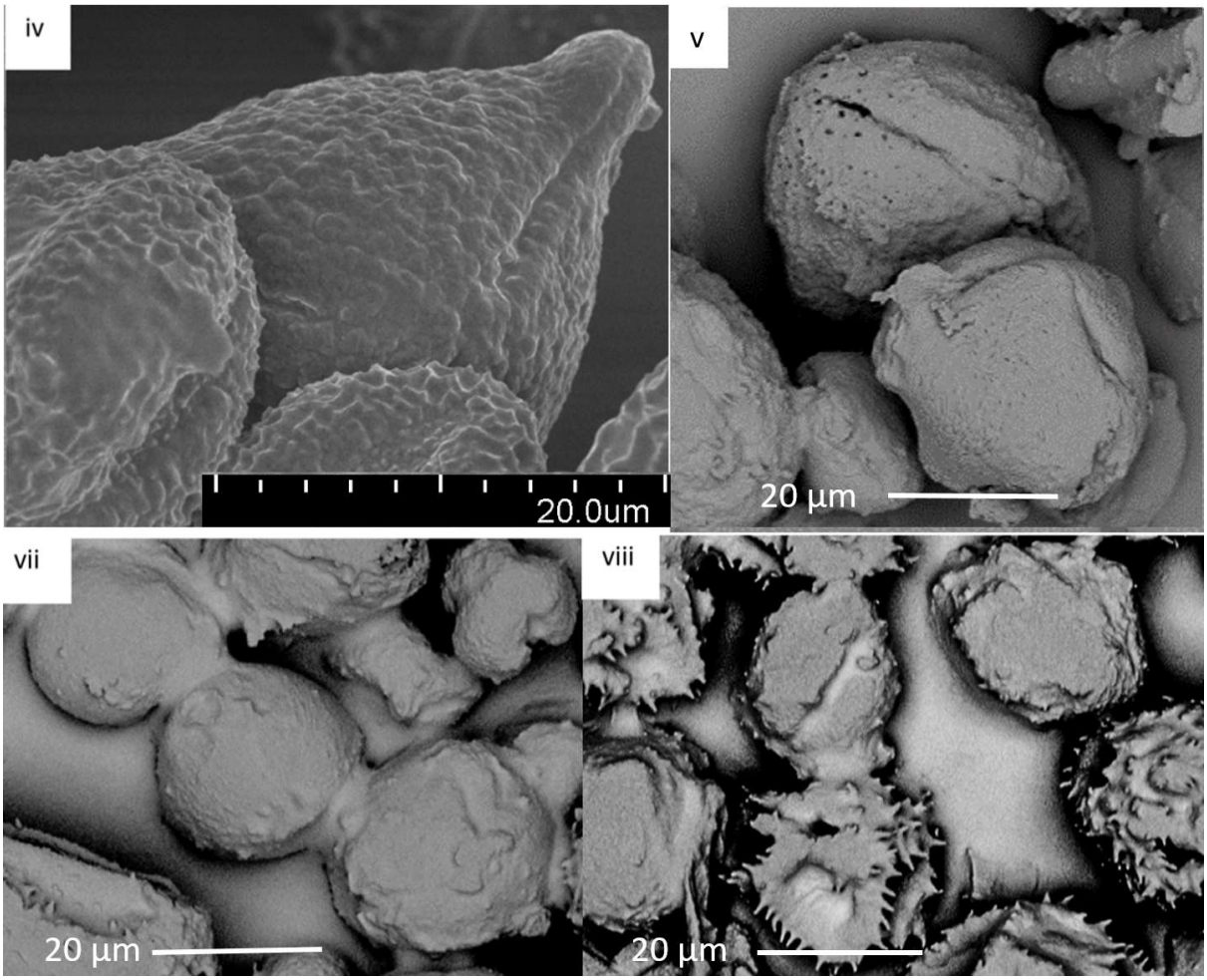


Fig 2.15. Pollen types IV (oval with pinched ends, slightly bumpy texture), V (smoother, deep furrows, rounded octahedron), VII (smooth, rounded), and VIII (deep indentations edged with long spines) identified from pollen provisions of *Halictus rubicundus* recovered from excavated nests at site 1 in May, 2015. Pollen type VIII are Asteraceae – Liguliflorae, possibly *Taraxacum officinale*.

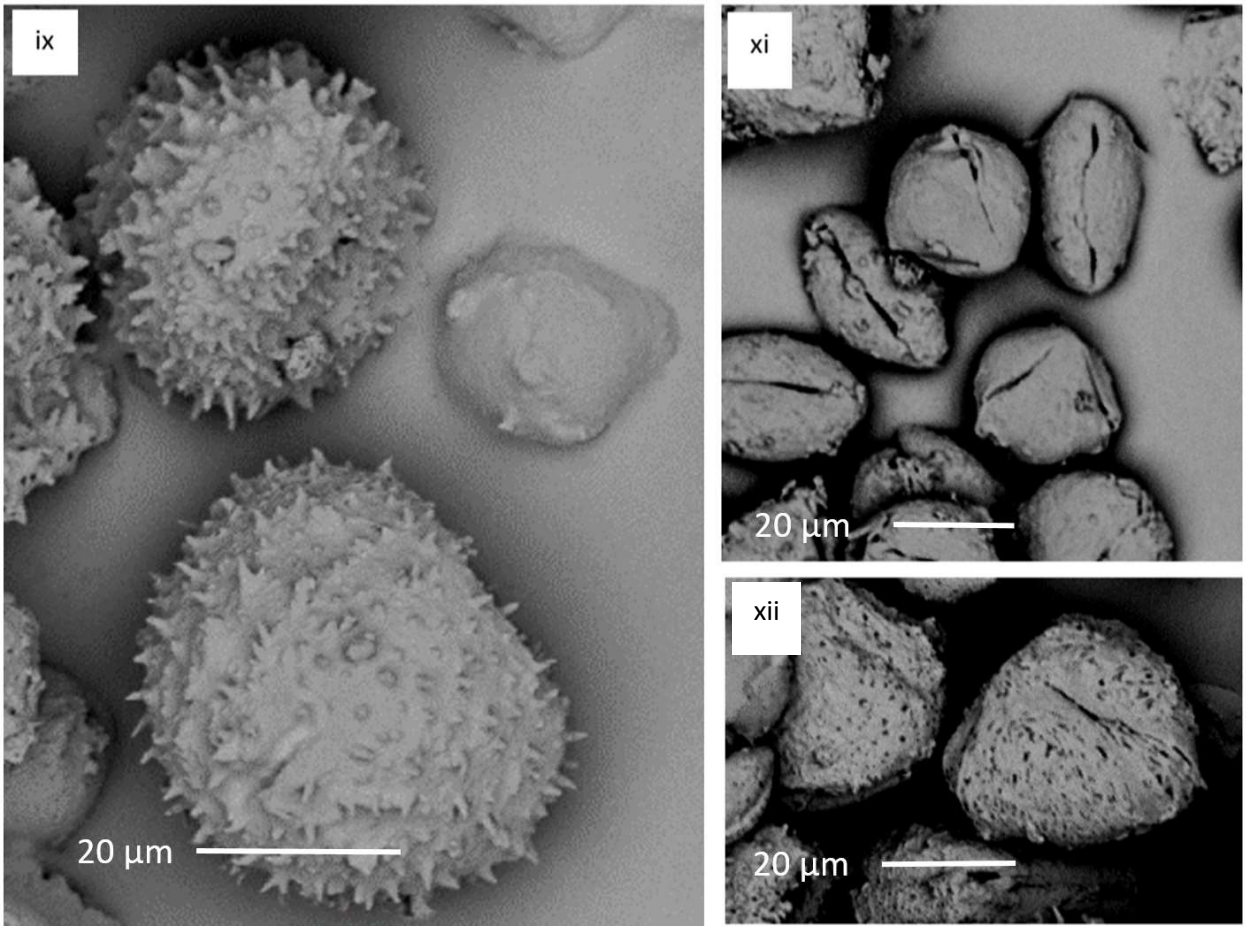


Fig 2.16. Pollen types IX (rounded pollen covered in somewhat equal, short spines), XI (smooth, three furrows with wavy edges, oval), and XII (flattened and triangular with pitted exine patterning) identified from pollen provisions of *Halictus rubicundus* recovered from excavated nests at site 1 in May, 2015.

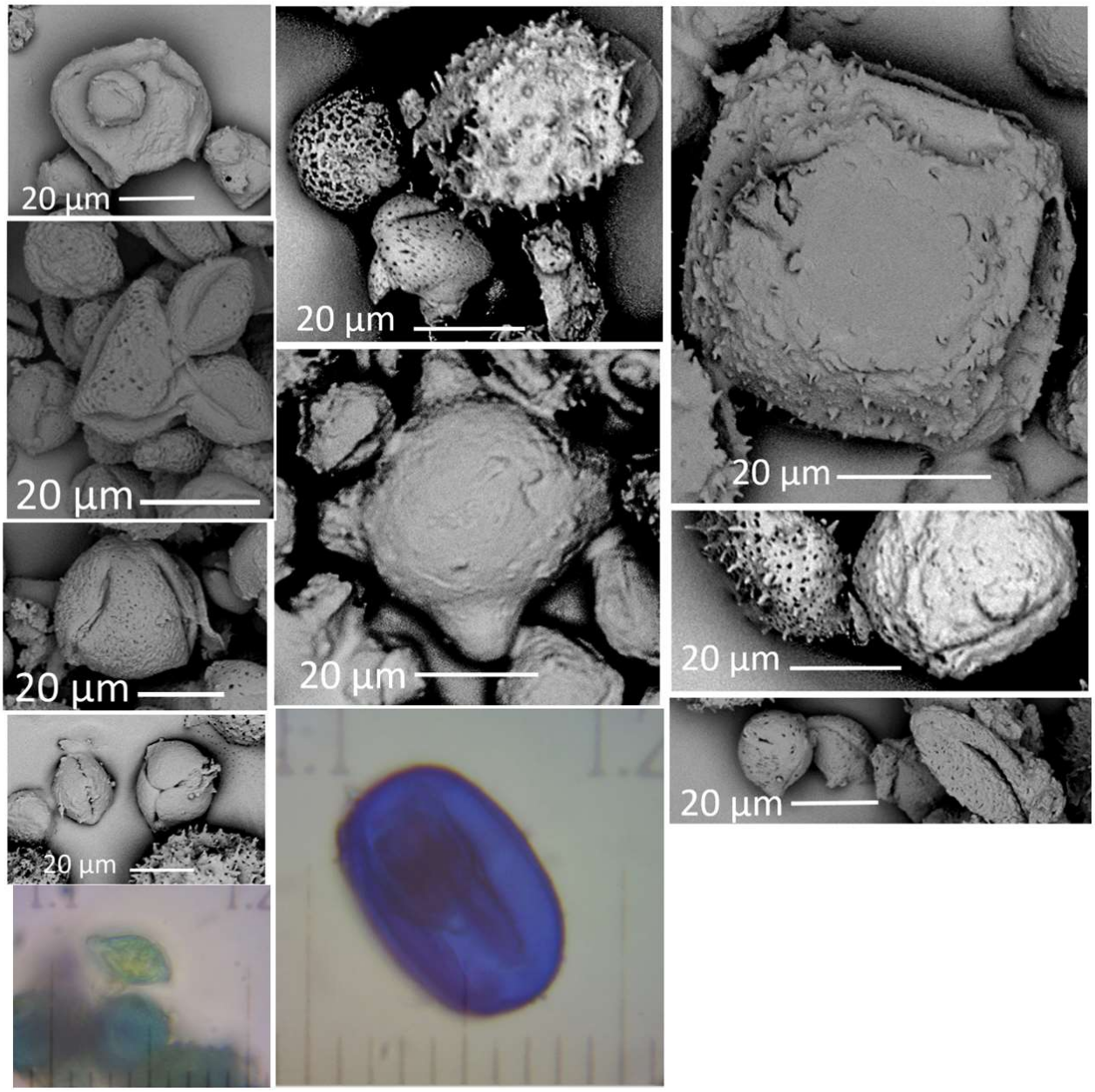


Fig 2.17. The less commonly encountered miscellaneous pollen types from pollen provisions excavated from nests of *Halictus rubicundus* at site 1 during May and June, 2015. The SEM images are all from the eight pollen provisions destructively subsampled for quantification of pollen (see Table 2.3) and generally represent less than 1.9% (rarely, up to 7.2% in Ps7) of numbers of the pollen grains identified as distinct types. Light microscopy images also represent the rarer pollen grains located, but were not meaningfully quantified.

2.4 Discussion

2.4.1 Characteristics of nesting biology of *Halictus rubicundus*

Nests of *H. rubicundus* were generally small and with few differences from nests described previously in the literature. The foundresses excavated early in the year, constructing new cells as they moved downward, with those early season cells constructed relatively close to the surface (Fig 2.6d). Both of the nest aggregation sites in this study were physically similar, suggesting there may either be something attractive about the how the sites are organized (e.g., large rocks recessed in barren dry dirt), or, considering that this species is described as philopatric and return to the same site annually (Yanega 1990), how the sites used to look, but there is no information on the age of the nest aggregation. The proximity to large rocks may provide some benefit, such as temperature maintenance or nest structure. Some of the nests follow the curvature of the stones downward before becoming more vertical, and it may be that nests constructed this way are more structurally secure or possibly just easier to build, requiring less digging and removal of soil. As part of a larger comparative study looking at the behaviour of members of Halictinae within their nests, females of *H. rubicundus* were induced to nest between transparent sheets of Plexiglas and provided with pollen (i.e., *Salix*, *Alnus*, and *Helianthus*) (Batra 1968). Three nests were constructed and were described as proceeding similarly to the nests built by *Lasioglossum* spp., also observed during the study. Some of the findings in regards to *H. rubicundus* specifically, helpfully summarized and noted as being unusual by Roberts (1973), were that female *H. rubicundus* did not close brood cells, instead opting to periodically inspect the cell contents (pollen provisions, eggs, and developing larvae). This report is contradictory to what was found at site 1 and site 2. The reasoning behind pouring plaster of Paris down the nest tunnels was to trace the nest without damaging nest contents and there was no indication that plaster of plaster of Paris ever entered the brood cells. The cells maintained integrity even after being excavated and appeared to be sealed off from the tunnel (Fig 2.6a,d,e). In addition, Batra (1968) found that females, presumably unrelated, would move into the same nest and cooperate to construct and provision the nest, with one female occasionally aggressively guarding the cell contents from the other inhabiting female. This type of behaviour was never directly observed during excavations. However, this finding by Batra could explain the presence of multiple females per nest early in May (Fig 2.8). On May 7, 2015, two adult females were observed in the same nest (N1), and each of the nests N1 and N4 on May 13 also had two females present.

Multiple adult females per nest early in the field season would imply that two overwintered females were possibly cooperatively constructing and provisioning the same nest based on work by Batra (1968). However, since the females were not observed interacting with each other, the interaction could be an antagonistic one, such as intraspecific nest usurpation. Roberts (1973) advised looking for the preceding behaviours in natural systems as they were observed originally under experimental conditions.

Differences in adult body size among adult female bees within a species can result from the amount of food provisioned, with certain eggs receiving preferentially more food (Michener 2007). However, differences in size result from other factors and may also reflect the degree of sociality. Other studies have measured variation in body size of adult female and male bees of *H. rubicundus*, but have measured such variation using wing length, and as such, are not directly comparable to observed differences in body mass (2.3 – fold among females; 1.7 – fold among males; 1.5 - fold between the sexes) except in the most general sense (Yanega 1989; Field et al. 2012). There was little evidence of protandrous emergence or of a sex-biased ratio as nests of *H. rubicundus* were not observed for a long enough period in order to determine whether the males had left or were never present. With the exceptions of nests N1 and N2 on June 1, N1 on June 9, and N1 on June 16, many of the nests evidently had a low number of total inhabitants per adult female bee. There were 14 cells associated with a single adult female bee on June 1, 2015. Whether she constructed those nests by herself or previously had a worker's assistance is unknown. For comparison, in New York State, Yanega (1989) reported that the mean productivity in the second brood was 1.5 individuals per worker. In Colorado each solitary foundress averaged 6.5 brood cells per nest (Eickwort et al. 1996).

A previous study that focused on wild bees visiting apple (*Malus* spp.) took place in Nova Scotia, Canada and makes mention of *H. rubicundus* with a description of the bee's life history in the area (Atwood 1933). Atwood described *H. rubicundus* as nesting in various types of soil, chiefly where it was dry and firm with sparse ground cover. The nests themselves were described as tunnels that were simple and relatively straight, seldom crooked or branched, that rarely exceeded 20.5 cm and with entrances approximately 6.4 mm in diameter that widened slightly inside. Adult females of *H. rubicundus* began their nests in early May, with complete cells found around May 20. The first female progeny emerged in the middle of July, and the males emerged

around two weeks afterwards. Both males and females continued to emerge well into August. Males were found on flowers drinking nectar, or waiting in holes or in sunny spots for passing females, and remained in the area until killed by frost. The males collected during nest excavations were apparent nest inhabitants and occasionally observed performing what appeared to be the same guarding behaviour as females. Yanega (1990) observed that a minimum of 31.5% of the males in a nesting aggregation in New York returned to a nest at some point, and estimated that 70% or more of males would return to nests. The nests entered were not always the male *H. rubicundus'* natal nest, but the males did not often encounter resistance from the nest inhabitants (Yanega 1990).

The same nesting preference for ground with sparse or absent vegetation was observed at site 1 in this study, wherein one part of the rock garden was noticeably more cluttered with plant debris, whereas the barren region had nest entrances of 3-4 mm in diameter (Fig 2.1). It is unknown when the first female progeny emerged, but is estimated around June 16, due to the increase in multiple females per nest, and adult male *H. rubicundus* appeared in mid to late June (Fig 2.8). During August, the well populated nests became scarce and the number of adult bees found at site 1 drastically decreased. The last male *H. rubicundus* at site 1 were observed in mid-September, 2015.

In this study, evidence of brood cell provisioning was apparent in early May (Fig 2.8), with the first larvae also found in early May. The lack of larvae found until May 30, a date that coincides with the first pupae found in nest excavations, indicates that some of the females within this population were laying eggs earlier than might be expected based on the data available for early May. There seemed to be little if any indication that cells were reused, with cells later in the season being deeper, and the inhabitants of the shallower cells often being visibly more developed (in larval size or pupal sclerotization). Adult females were the first bees observed, both within and outside of nests, and their numbers were relatively steady throughout the season (Fig 2.8), with no major peaks or die-offs. Overall, there were more females than males produced, but some of these females presumably immediately mated and left to overwinter. Other females may have faced higher mortality rates due to foraging and nest maintenance, which could also explain why there is not a larger peak of adult females following the large number of immature females observed in early June (Fig 2.8). The major peak of larvae in early June seems to be primarily

female judging by the echoing peak in mid-June. This female-biased first brood is similar to what is reported in the literature, however, it should be noted that in previous descriptions of the species, male *H. rubicundus* are often observed outside the nests and, hence the lack of adult males until mid-June could just be reflecting this behaviour. The presence of pharate males in late May is somewhat unexpected, due to the appearance of early stage female pupae, but could be explained as an alternate mating strategy. For example, an unmated female could begin laying her own unfertilized eggs instead of assisting her mother. Being unmated, her eggs remain haploid because of the lack of fertilization, and can only yield males. The number of males appears to fluctuate, but the proportion of males outside the nests is unknown. The males that did inhabit nests sometimes appeared to guard in the same way females did.

Roberts (1973) describes *H. rubicundus* as primitively social (one foundress that performs all tasks until daughters can assist) or communal (two adult females in the same nest), and both of these seem to be present even in early spring, with nests being inhabited by one or more females, although the condition of division of labour cannot be claimed without direct observation. Some female *H. rubicundus* were observed guarding, but very rarely was a returning foraging female seen returning to a nest. Roberts (1973) also excavated nests in Idaho (August 5, 1970) of approximately 20-25 cm depth (as compared to 10-50 cm in this study), containing adults of both sexes, and observed nest guarding (without sex of guard specified). Guarding has been described as one of the maintenance duties of the workers of *H. rubicundus*, the non-gynes (females who may reproduce, but do not undergo diapause), and as a principal duty of the "queen" (Soucy 2002).

It is probable that some of the *H. rubicundus* are displaying solitary behaviour at site 1, because there were multiple nests throughout the year that had a solitary adult female present. However, this conclusion could reflect when the nests were sampled, with the foraging daughters possibly having died. Without progressive observations of a single nest, it can be difficult to determine the level of sociality present. It has been noted that the socially plastic *H. rubicundus* is social in warmer climes, solitary in cooler climes, and can show mixed behaviour in certain environments (Soucy 2002).

The nests of solitary and social *H. rubicundus* are illustrated by Soucy (2002), being very similar in their basic structure: a vertical tunnel with a few cells in a cluster branching off

relatively close to the surface, at around 3 cm, whereas the social nests extend further downward, past 6 cm and have a separate cluster of cells at around 10 cm. With the exception of the clustering of brood cells, this is very similar to what was found at sites 1 and 2 in this study. If one takes the life estimate of two months for adult female bees at face-value (Batra 1968), then activity later in the season could be evidence that this Saskatchewan population at site 1 had two generations (a bivoltine population) because the whole field season exceeded three months, and it is very possible that the adult female *H. rubicundus* were active prior to field observations began in late April, 2015.

2.4.2 Pollen provisions within nests of *Halictus rubicundus*

The mean dry mass of 18 pollen provisions collected in May was 36.1 ± 9.7 mg. As there is an apparent lack of information in the literature regarding *H. rubicundus* pollen weights in the literature, other species of bees provide the only available comparisons. The solitary bee *Ceratina calcarata* had mean wet weights of 15.8 ± 4.1, 19.9 ± 5.5, and 17.9 ± 4.3 for 1983, 1984, and 1985 respectively (Johnson 1988). Notably, the author found a significant difference in the weights of pollen provisions provided to male versus female larvae, with females receiving a provision that was 1.3 times heavier than the provision the male received. This is reflected in the weight of the adults, with adult female *C. calcarata* weighing, on average, 1.3 more than males. The primitively eusocial bee *Halictus scabiosae*, was reported as having a dry weight of 77.5 ± 12.9 mg (fresh weight 125.5 ± 19.9) (n=16) for its first brood, and a dry weight of 112.3 ± 23.4 mg (fresh weight 177.4 ± 37.5) (n=16) for its second brood. (Brand and Chapuisat 2012). As all of the 18 pollen provisions that weighed were from May, the average of 36.1 ± 9.7 mg from this study very likely represents only first brood provisions. The provisions collected by adult female *H. scabiosae* appear to be twice as heavy as those collected by *H. rubicundus*. This may partially account for the size difference between the two species. Female *H. scaboisae* were reported as weighing 26.13 ± 4.26, 20.93 ± 3.80, or 25.94 ± 5.94, depending on whether they were foundresses, first brood females, or second brood females. The female *H. rubicundus* collected had an average mass of 13.09 ± 4.46, and were lighter in comparison. Male *H. scabiosae* had a reported average dry weight of 13.16 ± 4.34, whereas the male *H. rubicundus* had a mean mass of 8.53 ± 2.36.

The polylectic *H. rubicundus* had a wide variety of pollen types present as provisions within its subterranean nests. There were 12 pollen types recorded quantitatively and by micrography that fluctuated in frequency over time (Table 2.3), as well as various miscellaneous types (Fig 2.17) that were less common. The pollen types found varied not only in quantity, but also size and exine sculpturing, with several grains obviously belonging to Asteraceae, which is a well known pollen source (Soucy 2002). Several of the pollen grains appeared to represent members of Rosaceae, which is also well documented in the literature (Soucy 2002), as well as being anticipated from foraging observations made during the 2013 survey season. During this study, foragers of *H. rubicundus* were observed visiting *Prunus tenella* (identified by Sabine Banniza), an ornamental species present on campus, as well as dandelion, (*Taraxacum officinale*), very early in the year when nothing else was in bloom. Whether the bees that visited *P. tenella* and *T. officinale* during 2013 actually collected pollen during their visits is unknown. It is very possible that pollen type VIII is *Taraxacum officinale,* but many of the pollen types remain unidentified, with Types I, VI, VII, X, and XI possibly representing Rosaceae, with Type I arguably being the most important, at least for early spring provisioning. Therefore, at least some species of Asteraceae and Rosaceae appear to be predominant in the larval diet of *H. rubicundus* in Saskatchewan, as indicated earlier for *H. rubicundus* (Soucy 2002).

The very noticeable switch from homogenous to heterogeneous pollen provisions may have some interesting implications. In other bees, specifically *Osmia* (Megachilidae), an observed preference for Asteraceae pollen is thought to improve resistance against parasitic wasps (Spear et al. 2016). Asteraceae pollen is considered to be a low-quality food source, but significantly reduces survival rates of the cleptoparasite *Sapyga*. The increase in Asteraceae pollen grains was very obvious in the later part of the season, and coincided with the majority of observations of *Sphecodes*. It should be noted that in a previous year (2013), female *H. rubicundus* were observed foraging on dandelion relatively early. As the change from homogenous to heterogeneous provisions happened later in the year, it could also be a consequence of multiple females within a nest visiting different floral resources, or just a reflection of increased choices of floral resources as the season progressed. Two pollen provisions from the same nest, Ps7 and the incomplete Ps8 (N1 June 1, 2015), which displayed markedly different pollen profiles, were possibly provisioned by the same female (i.e., only one

adult female was found in N1). If so, that individual female of *H. rubicundus* had made a switch in choice of pollen types for her progeny's provisions.

CHAPTER 3 - THE HISTIOSTOMATID MITE, *ANOETUS HALICTONIDA* [WOODRING] (SARCOPTIFORMES: HISTIOSTOMATIDAE)

3.1 Introduction

Associations between mites and bees are well documented, and may involve a diverse assortment of mite life histories, ranging from parasites to scavengers to phoronts (Eickwort 1994). Within this multitude of strategies employed by the Acari, those of the Astigmatina (=Astigmata) show some distinctive traits. In astigmatids, it is specifically the deutonymphs (as opposed to the female adults in certain other Acari) that have evolved the behavioural and morphological characteristics to facilitate phoresy including the loss of the mouth and chelicerae, the reduction of the gnathosomal remnant (sometimes called the palposoma [Fain and Erteld 1998]), the occurrence of a solid and non-functional gut, a dorsoventrally flattened and sclerotized body, and modified legs (shorter, stouter, and with modified chaeotaxy or arrangement of bristles) (OConnor 1982). It has been observed that deutonymphs are typically very active, and certain taxa are attracted to chemical cues from hosts, although deutonymphs can be induced to attach to most moving objects (OConnor 1982). Deutonymphs can recognize whether a host is appropriate after initially attaching, by either moving to a particular site on the body, or by disembarking. Indeed, deutonymphs of some species, such as *Ensliniella trisetosa*, can recognize the sex of their host, *Ancistrocerus antelope*, and attach preferentially at sites on the male body that are in contact with the female during copulation to facilitate deutonymph transfer (Cooper 1954).

Within the Astigmatina, the superfamily Histiostomatoidea (=Anoetoidea) displays the "entomophilous" form of deutonymph, assumed to be ancestral within Astigmatina, with its extensively developed suckers and common attachment to arthropods (OConnor 1982). These mites have a haplodiploid sex determination, allowing a single female to found a colony via parthenogenesis. In comparison, in bees, which also possess a haplodiploid system of sex determination, the foundress females that construct new nests and produce the subsequent generation are understood to have mated in the previous season. Both arrhenotoky and thelotoky, different forms of parthenogenesis, are present within Histiostomatidae (Hughes and Jackson 1958). Arrhenotoky refers to when males develop from unfertilized eggs and females from fertilized eggs, whereas in thelotoky, females develop from unfertilized eggs. Histiostomatid

mites are described as devoted filter feeders, with modified chelicerae (when chelicerae occur) and a tendency to be found living on surfaces with liquid films (Eickwort 1979). Deutonymphs of *Histiostoma laboratorium* have been reported to use the posterior pairs of legs to jump approximately one to two inches (Hall 1959). Jumping could be induced and was posited as a way mites could attach to insects in flight. The deutonymphs are thought to specifically use legs iii to accomplish this behaviour and the mites had a common "pre-jumping" position in which the mite's body was tilted and the anterior end was elevated.

Within Histiostomatidae, the genera *Anoetus* and *Histiostoma* are most relevant to this chapter. These two genera are very similar, with several *Histiostoma* later reassigned as *Anoetus*. This reclassification is due to Mahunka defining *Anoetus* in a 1974 paper as obligatory associates of halictine bees (Eickwort 1994). *Anoetus* are very common in halictine nests, being encountered worldwide (Eickwort 1994). Mites of *Histiostoma* show similarities to *Anoetus* in development and appearance, but have been described (along with the very similar members of the genus *Glyphanoetus*) as "catch-all genera" and occur on other bee taxa (i.e., Nomiinae, Anthophorinae, Xylocopinae) (Eickwort 1994), whereas *Anoetus* species are more limited in their host range.

The name *Anoetus halictonida* (Woodring, 1973) was agreed upon by Drs. Klimov, OConnor, and Proctor after examining SEM photos of deutonymphs collected from site 1 in 2015. The reclassification from *Histiostoma* to *Anoetus* is based on a paper by Mahunka (1974), who grouped together all mites of Histiostomatidae associated with halictids together within the genus *Anoetus*. The mite *Histiostoma halictonida* was not mentioned in Mahunka's paper, possibly because Mahunka's revisions came only a year after Woodring (1973) had published a description of that mite. The mite found in this study is very similar to the original description of *H. halictonida*, the only major difference being the original description of the deutonymph lacked reference to a characteristic dorsal pattern. This difference was noted by Dr. OConnor as possibly suggestive of the age of the specimen used, and he desired to view the paratype in order to determine if the deutonymph was recently eclosed. Dr. OConnor also examined 3 deutonymphs from a subterranean nest of *Halictus rubicundus* [Christ] (Hymenoptera: Halictidae) collected from Ithaca, New York (on loan from Cornell University) by the late Dr. G. Eickwort, plus reviewed other deutonymphs in his possession taken from nests of *H. rubicundus* in Iowa, Kansas, and Utah, U.S.A., and England. In all instances, Dr. OConnor found the dorsal patterning

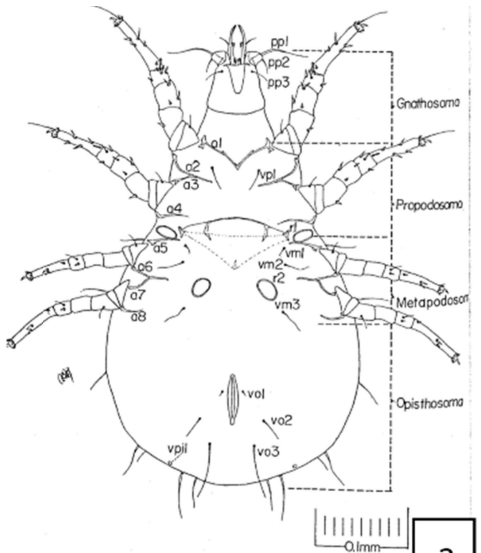
present on those deutonymphs matched that in the photos of the Saskatchewan mites sent to him and has concluded that they are probably all conspecific. The morphological features that match Woodring (1973)'s description are the developed coxal suckers, and the lyre-shaped concavity at the midanterior apodemes III, which are not arched (noted by Dr. Klimov). During the process of identifying this mite from Saskatchewan nests of *H. rubicundus*, Drs. Klimov and OConnor also disclosed some features for recognizing *Anoetus*: a gnathosomal remnant that is quadrate to trapezoidal, although some *Histiostoma* have similar gnathosomal remnants, and the shape of claws iii and iv (either small and thin as in *Anoetus* or large and hooked as in *Histiostoma*) (P. Klimov, B. OConnor, personal correspondence).

Histiostoma halictonida was described from specimens taken from nest cells of *Halictus rubicundus* located in New York. The collected stages were adult females (6), protonymphs (unknown number), deutonymphs (unknown number), and a lone tritonymph. It is unknown precisely how many of each stage were present, with the number of protonymphs and deutonymphs simply being listed as "many" (Woodring 1973). Included in the original paper by Woodring are the following notes by Dr. Eickwort describing the life history of *H. halictonidus* [sic] based on the contents on a single nest of *H. rubicundus*:

Biology—Dr. Eickwort provided the following notes on the biology of *H. halictonidus*. These observations are based solely on 1 *Halictus rubicundus* nest dug on 13 August 1969, and on microscopic and field observations on the contents of 11 cells brought into the laboratory in individual vials. Two of these cells contained mature bee larvae which were reared in plaster of Paris-charcoal lined dishes along with remnants of their cells and the associated mites. The contents of one other cell, in which the mites were feeding on the bee feces, were maintained and observed even though the bee pupa died on the first day and was removed. The phoretic deutonymphs presumably leave the adult female bee while she is provisioning a cell. The deutonymph presumably apolyses into a tritonymph and then into an adult during the time that the bee egg develops, hatches, and the bee larva consumes the provisions. The one cell examined that had contained a half-grown bee larva failed to produce any mites, and what the mite feeds on during this period is not known. At the time that the mature bee larva is beginning defecation, an infested cell contains up to 3 swollen adult female mites which slowly move on the cell wall. A female mite may contain up to 4 well-developed eggs at one time. Many mite eggs are stuck to the cell wall in small clusters of up to 5 eggs per cluster. In the cell with the 3 females over 50 larval mites were present. The larvae may occur on the bee prepupa in small numbers but rarely occur on the bee feces or on exposed dirt that is not covered by the secreted cell lining. At about the time the bee is ecdysing its larval cuticle, the mites are beginning to apolyse into protonymphs. The protonymphs move onto the bee pupa in large numbers. They are especially abundant in the ventral regions of the head and thorax of the bee pupa, and they presumably feed while on the bee. Within 2 days of pupal development, many of the protonymphs are actually pharate deutonymphs. Active deutonymphs begin to appear about 5–7 days into the pupal stadium of the bee, and most (although not all) mites on the pupa are active deutonymphs by 11 to 12 days into the stadium. The older deutonymphs cluster dorsolaterally on the propodeum and metanotum of the pupa, in the region of the large propodeal spiracles. Up to 8 deutonymphs clustered on one side of a pupa, and they attach firmly with their caudal suckers. As the bee ecdyses its pupal cuticle, the hypopi presumably leave the exuvium and transfer to the emerging adult bee, walking on the base of the wings and the propodeum. They attach preferentially to the undersurfaces of the front wings. This phoretic position is important because the bee undergoes extensive grooming before leaving the cell; it carefully cleans the upper surfaces of its wings but cannot reach the undersurfaces.

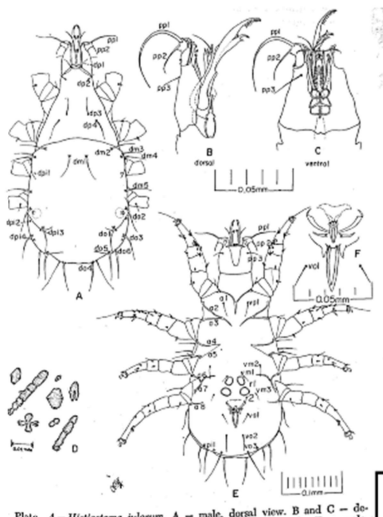
As a synopsis from Eickwort's observations published in Woodring (1973), it is presumed the phoretic deutonymphs disembark from the adult female bee while she provisions the cell, and then develop into adults while the immature bee develops simultaneously. When a bee larva begins defecation, there are up to 3 gravid adult females present on the nest cell wall, each containing a maximum of 4 eggs, which will be fixed to the cell wall in clusters. Large numbers of larval mites may be present in a cell, occurring on the bee prepupa in low numbers, but usually not on the feces or on dirt, only on the secreted cell lining. When the bee is ecdysing the larval mites apolyse into protonymphs and move onto the bee, often onto the ventral regions of the head and thorax. The mites are presumed to feed while on the bee. Active deutonymphs appear in about a week and most of the mites are active deutonymphs within about 12 days. These deutonymphs are located dorsolaterally on the pupa's propodeum and metanotum, in addition to the sides of the pupa, securely attached with their caudal suckers. It is presumed that during the time the bee ecdyses, the hypopi transfer from the exuvium, walking on the wing and propodeum bases. The mites preferentially attach to the undersides of the front wings. The bee grooms extensively before leaving the cell, but cannot properly clean the lower surfaces of its wings. It was noted that the mites could survive on the bee feces in the case of the death and removal of a bee pupa.

It is unknown what the minimum time requirements are for the completion of the life cycle of *Anoetus halictonida*, but the species *Histiostoma julorum* managed to complete its life cycle in six days under optimum conditions (Hughes and Jackson 1958). Hughes and Jackson provide one of the most complete life cycle descriptions of a histiostomatid mite in their review (1958), with the different nymphal stages and sexes of *Histiostoma julorum* given here in detailed overview (Fig 3.1a-e).



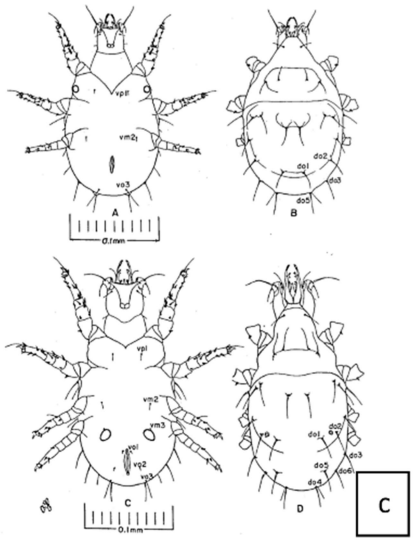
a

Plate 1 — *Histiostoma julorum* female. Ventral view.



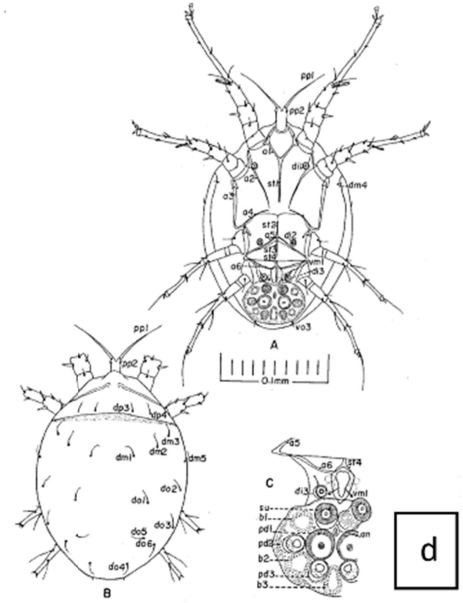
b

Plate 4 — *Histiostoma julorum*. A — male, dorsal view. B and C — details of gnathosoma. D — typical body crystals. E — male, ventral view. F — details of external genitalia.



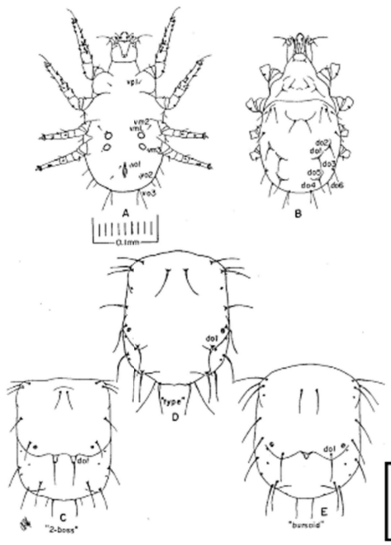
c

Plate 6 — *Histiostoma julorum*. A — larva, ventral view. B — larva, dorsal view. C — protonymph, ventral view. D — protonymph, dorsal view.



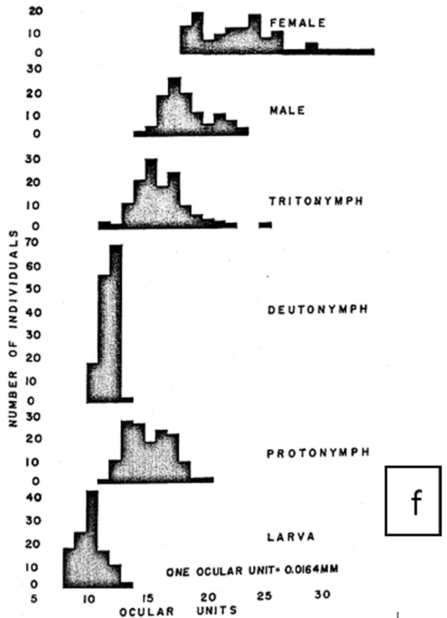
d

Plate 7 — *Histiostoma julorum* deutonymph. A — ventral view. B — dorsal view. C — details of suctorial plate.



e

Plate 9 — *Histiostoma julorum*. A — tritonymph, ventral view. B — tritonymph, dorsal view. C, D, E — males, dorsal hysterosoma of the three "varieties" — "type," "bursoid," and "2-boss."



f

Plate 13 — *Histiostoma julorum* — size range of various stages.

Fig 3.1. Visual summary of the life cycle stages of a typical histiostomatid mite, *Histiostoma julorum*. Life stages described in review by Hughes and Jackson (1958): a) female, b) male, c) larva and protonymph, d) deutonymph, e) tritonymph types, as well as a size comparison of the life stages f).

Whereas Woodring (1973) lacks a description of immature stages of *Histiostoma halictonida* with the exception of the deutonymph, that paper does describe females of the species (males were lacking in samples collected by Eickwort). Many of the existing keys rely primarily on the characteristics of deutonymphs, and sometimes on the adult forms. This reliance on deutonymphs can be partly attributed to what is available, as some species are only known by the deutonymphs. In comparison to the other nymphal stages and even the adults, deutonymphs are distinctive, with adults from some species lacking clear characteristics that could be used to differentiate between species (Hughes and Jackson 1958). For a typical species within Histiostomatidae (=Anoetidae), *Histiostoma julorum* was described as having protonymphs (Fig 3.1) and tritonymphs very similar to adult (non-gravid) females, miniatures with some characteristics differing. It must be noted that every developmental stage of this typical anoetid mite showed great variation in size, with the size ranges of the protonymph overlapping those of the tritonymph, which in turn had several representatives of similar size to adult females (Hughes and Jackson 1958). Because of this range in body size (Fig 3.1f), it would not be unreasonable to assume that immature stages excluding the deutoynmph may be difficult to distinguish.

There is some information about how many *Anoetus halictonida* can be found on *Halictus rubicundus*, but there is a lack of data beyond total numbers of mites and the general body regions occupied. The mite *A. halictonida* (reported as *H. halictonida* in the paper), was found on a species of *Megalopta* (see Table 1.1) in Central America (Engel and Fain 2003). Mites were found on 20% of the adult halictids, sex unspecified, in numbers ranging from 1-72 mites per bee. These particular mites were located anteriorly on the first metasomal tergum and on the extreme wing bases in the absence of an acarinarium. There is little quantitative information on which areas of the host body have comparatively high mite loads or if there is any temporal variation. In addition, the original studies on *Anoetus halictonida*, can benefit from more recent re-evaluations given that the species does not seem to be reclassified. Another advantage is the improvement of SEM, which provides increased resolution and more detailed information about the fine structural features of the mites, potentially improving the ability to distinguish between mite species. It is generally assumed that *A. halictonida* and related mites are phoretic (occasionally hypothesized to be mutualistic based on their feeding habits) and cause little to no harm to their hosts, and this is not an unreasonable possibility, since several definitions of hypopi make sure to emphasize their non-feeding state. However, a lack of direct harm inflicted on the

host, e.g., parasitism, does not prohibit indirect effects on fitness and an attempt should be made to determine the effect of mite load on the host. There is very little information on how these mites move and transfer, with much of the presumed behaviour acquired from observations of other hymenopterans. Whether the mites transfer between individual hosts and between bee species, and how precisely this occurs, is somewhat unclear.

Therefore, following an inaugural summer of research (2013) as a senior undergraduate involving capture of foraging adult bees of various species and preliminary observations of any phoretic mites, this graduate study conducted over two consecutive field seasons (2014, 2015) that included excavations of the subterranean nests of *Halictus rubicundus*, addressed the following objectives by

a) furthering our understanding of the biology and life cycle of *Anoetus halictonida*;
b) examining the mechanism of attachment, relative abundance on the body of both female and male hosts, and ability of immature mites of *A. halictonida* to transfer among adult bees; and
c) providing an estimate of the phoretic load of immature mites of *A. halictonida*.

Building upon previous work in other North American locations such as New York state (Yanega 1990) and Colorado (Eickwort et al. 1996), the studies described below evidently mark the first investigations of the relationship between *H. rubicundus* and *A. halictonida* undertaken in Canada.

3.2 Materials and Methods

3.2.1 Mite distribution within excavated nests and on inhabitants

3.2.1.1 Mites on adult bees

Halictus rubicundus bees collected during the 2015 excavations at site 1 (see 2.2.1) were stored in individual vials in the freezer before viewing. Individual bees were caught as they exited the nest when they often stopped to groom the plaster of Paris utilized to detect nesting tunnels prior to excavation (see Section 2.2.2). Vials were upturned to trap adults before being sealed and set aside as excavations continued. At the lab, the bees were viewed on a section of cork under a dissecting microscope and rotated to allow for an unimpeded view. Bees were not

pinned due to concerns that the pins would cause damage to the bees and could accidentally remove some of the adhering mites. However, dissecting needles and pins were used to manipulate specimens (e.g., moving the wings, pulling back hairs of the scopa) during their examination for mites.

3.2.1.2 Mites on immatures

All immature bees examined were those individuals collected during nest excavations at site 1 during the spring-summer 2015 field season. All life stages found within the cells, namely the eggs, larvae, pupae, and pharate individuals (pharate referring to those bees that had metamorphosed into adults without exiting the exuvium or leaving the cell), were placed in their own vials and stored in the freezer (-3°C) until examination later in the lab.

3.2.1.3 Mites within brood cells

Examination of the brood cells for mite presence was complicated due to the destructive nature of the procedure. Intact pieces of cell wall lining, generally the lower walls, were viewed under a dissecting microscope for the presence of mites, with certain pieces of the brood-cell walls viewed later with SEM.

3.2.2 Mite location on bees

3.2.2.1 Mite distribution on adult female bees

The mite distribution for each adult female *Halictus rubicundus* refrigerated in separate vials after collection during site 1 excavations was determined using a dissecting microscope on a cork board as described in section 3.2.1.1. Mites in this particular portion of the project were left in their original areas and orientation on the adult bee body. Mites that were accidentally removed and recovered were either placed in vials or repurposed.

The percentage of the grand total of 2,870 mites (all deutonymphs) of *Anoetus halictonida* found in particular regions of the adult female's body (n=52 of the 54 bees possessed mites) were mapped onto schematic drawings of the host. Each body region was indicated by a black line boundary, a number indicating the total percentage of mites found, and a colour denoting the relative frequency of deutonymphs recorded. Colours were assigned based on bins with higher percentages represented by darker colours, and the complete absence of mites indicated by white.

Areas were assigned by viewing multiple females and detecting areas that were consistently occupied by any mites. Not all female adult bees had mites in all indicated body regions, but certain areas were especially well populated and were classified separately despite being adjacent to other areas.

The adult female bees collected during 2015 (n=54) were examined further based on a previous study (Cross and Bohart 1969) that had investigated whether mite loads on host bees were symmetrical, by quantifying the mite loads of *Imparipes americanus* on *Nomia melanderi*. To determine whether there was a bilateral symmetry in mite loads of *A. halictonida* on the host body of *H. rubicundus*, a pooled Chi-square test was performed to compare mite loads on the left and right forewings and hindwings, with the null hypothesis being that there was no significant difference at P=0.05. Only adult females of *H. rubicundus* were used due to their differences in distribution of mite loads, and only the wings were analyzed as they are the most obvious body regions that can be examined separately into a distinct left and right, whereas many of the other regions of mite occupancy lay centrally along the host's longitudinal body axis (see Results). Cross and Bohart (1969) made a similar choice, only quantitatively analyzing mites that were located in the subalar pits and mesonotal angles.

3.2.2.2 Mite distribution on adult male bees

Collection and subsequent handling and storage of adult male bees of *Halictus rubicundus* recovered during nest excavations at site 1 in 2015 were identical to the methods described in section 3.2.2.1 for female bees. Similarly, the proportional distribution of the grand total of 481 deutonymphs of *Anoetus halictonida* according to their average regional occurrence on the body of male *H. rubicundus* (n=16 of the 28 bees which possessed mites) was represented by colour shading as outlined in 3.2.2.1.

3.2.2.3 Mite distribution on immature bees

Bee eggs were given a cursory examination during removal from excavated brood cells. They were later viewed for a limited time (<1 minute) under a dissecting microscope for any adhering mites, and returned to the fridge. Similarly, bee larvae were also only removed from the refrigerator for a limited time during examination with a dissecting microscope to avoid larval rupturing. The vials that the bee larvae had been stored in remained of interest due to the

presence of adult gravid female mites that had either climbed off or fell from the stored larvae. A few bee larvae were partially destroyed when pieces of integument, selected for examination of the presence of mites, were removed for SEM. Pupae and pharate individuals were manipulated with dissecting needles and pins (see section 3.2.1.1). The exuvium of these advanced brood stages was not intentionally removed, thereby including any mites residing on the exuvium.

3.2.2.4 Method of attachment

To obtain unimpeded observations of their attachment organs, mites were prepared for high magnification SEM in one of two ways. In the first, an adult bee was secured to a cork board with pins that did not pierce any part of the bee, but maintained the host bee's position on the cork by applying pressure. Using a pin specifically for its fine tip, mites could be removed from the body of the bee and transferred to the sticky tab on an aluminum stub for SEM (see section 2.2.4). Removal of the mites differed depending on location on the bee body and whether the mites had already dried. Dried mites were always easy to remove. Mites residing on the host bee's hard exoskeleton, such as the propodeum and tergites, were more easily removed than those on the wings, which occasionally required the pin to be slipped under the mite body and rotated around the mite's caudal suckerplate to cause mite detachment.

The second method, which had the two-fold advantage of preserving relative positions of groups of mites and without requiring any mite to be twisted off and potentially damaged in the process, required the partial dissection of the host adult bee due to its full size being prohibitive for the SEM instrumentation available. Wings, leg segments, and other body regions were removed from host bees and usually placed separately on SEM stubs. In order to show ventral surfaces of deutonymphs, including their caudal suckerplates, bees were removed from the freezer and had their wings removed with scissors. The wings were placed lower surface down on the SEM tab and then removed, leaving behind most, but not all, of the mites on the wing still in the mites' original orientations. This method necessitated the use of bees that had time to dry so that mites would actually be removed when the wing was gently pressed against the adhesive tape on the SEM stub.

3.2.3 Mite stages and their morphology

Mite stages were viewed with light and scanning-electron microscopy (SEM), for photography. Both dissecting and compound microscopes were used and images recorded using the Dinocapture camera (see section 2.2.4). For those mites viewed with the compound microscope, permanent slides were made by clearing the mites overnight (10% KOH) and mounted in Canada Balsam softened by xylene.

SEM photos of mites were taken with three different instruments on the University of Saskatchewan campus depending on availability (see section 2.2.4 for methods). The three different microscopes used were the Geology Department's Joel SEM, the Phenom G2 desktop scanning electron microscope in the Biology Microscopy Resources Facility, and the FE-SEM-cold field emission scanning electron microscope (Hitachi SU8010) at the Imaging Centre in the Western College of Veterinary Medicine.

Whereas deutonymphs are comparatively simple to remove and study, other stages of *A. halictonida* were more prone to desiccation and associated deformations. Bee larvae, selected for the presence of mites on the integument, were destroyed due to the removal of integument with loosely adhering mites. The integument sample was removed with a scalpel and air-dried to avoid introducing water into the Phenom microscope.

3.2.4 Mite weights and phoretic loads

Weighing of mites of *A. halictonida* (all deutonymphs) was performed at the same time and on the same equipment as the bee weighing described in section 2.2.5. There were some minor changes due to the size disparity and the interference by static electricity. Mites removed from host bees had to be weighed by pooling, as individuals did not register on the microscale or only registered momentarily. Using an eyebrow hair affixed to a dissecting needle with nail polish, deutonymphs were transferred to a tared thin sheet of aluminum. Static electricity was minimized somewhat by using a dryer sheet and by releasing charge by touching the tip of the eyebrow brush to a lightly damp paper towel and letting it dry. The average weight of deutonymphs was calculated and multiplied by the average mite load per bee (see section 3.3.1). This average mass was then expressed as a percentage of average dry weight of *Halictus*

rubicundus (see Table 2.2 of section 2.3.4), to determine the proportional burden of a phoretic load of *A. halictonida*.

3.2.5 Transfer of mites

3.2.5.1 Incidence of mite transfer to flowers

A preliminary project conducted during the spring-summer of 2013 involved collection of bees within and on the outskirts of Saskatoon, Saskatchewan, as they (see Appendix 2), and other insects foraged, and the collected insects were examined for mites. If a bee was observed on a flower or certain type of inflorescence, the flower or inflorescence was collected and fixed in labelled vials of 70% ethanol to be examined at a later date. Flowers were stored in individual vials at room temperature, before dissection under a microscope to expose any mites that had potentially transferred from bees to the flowers they visited.

Initially bees were euthanized with ethyl acetate, but later on by freezing them. It is unknown if this change in technique could affect the ability of mites to remain attached or if either method resulted in any change to the total numbers of mites recorded. In later field seasons (2014, 2015), only freezing was used. Mite counts were done without removing them from their host and while trying to keep specimens intact. Bees were identified to genus using the online DiscoverLife key (www.discoverlife.org/), the Bee Genera of Eastern Canada key by Packer et al. (2007), and the Bees of Northwestern America: Halictus by Roberts (1973). Bee sex was determined by counting the number of antennal segments, tergites or by distinctive traits.

3.2.5.2 Carbon dioxide anaesthetization

A brief attempt was made to see whether mites of *Anoetus halictonida* could be induced to transfer from one bee to another. There were several (n=7) attempts to anaesthetize bees with CO_2 on June 29 and July 9, 2015 to determine if the deutonymphs of this mite species could transfer between adult bees. Experiments involved pairing available adult bees in glass vials after anaesthetizing both and placing them not more than 4 cm apart. The two attempts in June consisted of one male-male pairing and one male-female pairing. The July attempts involved pairing males with females (n=4), and a female *Sphecodes* with a female *H. rubicundus* (n=1). Anaesthetization occurred by placing 5 dry ice pellets into the CO_2 chamber (i.e., water bottle) (Fig 3.2) which was connected to the holding chamber (the flat plastic dish) via PVC pipe

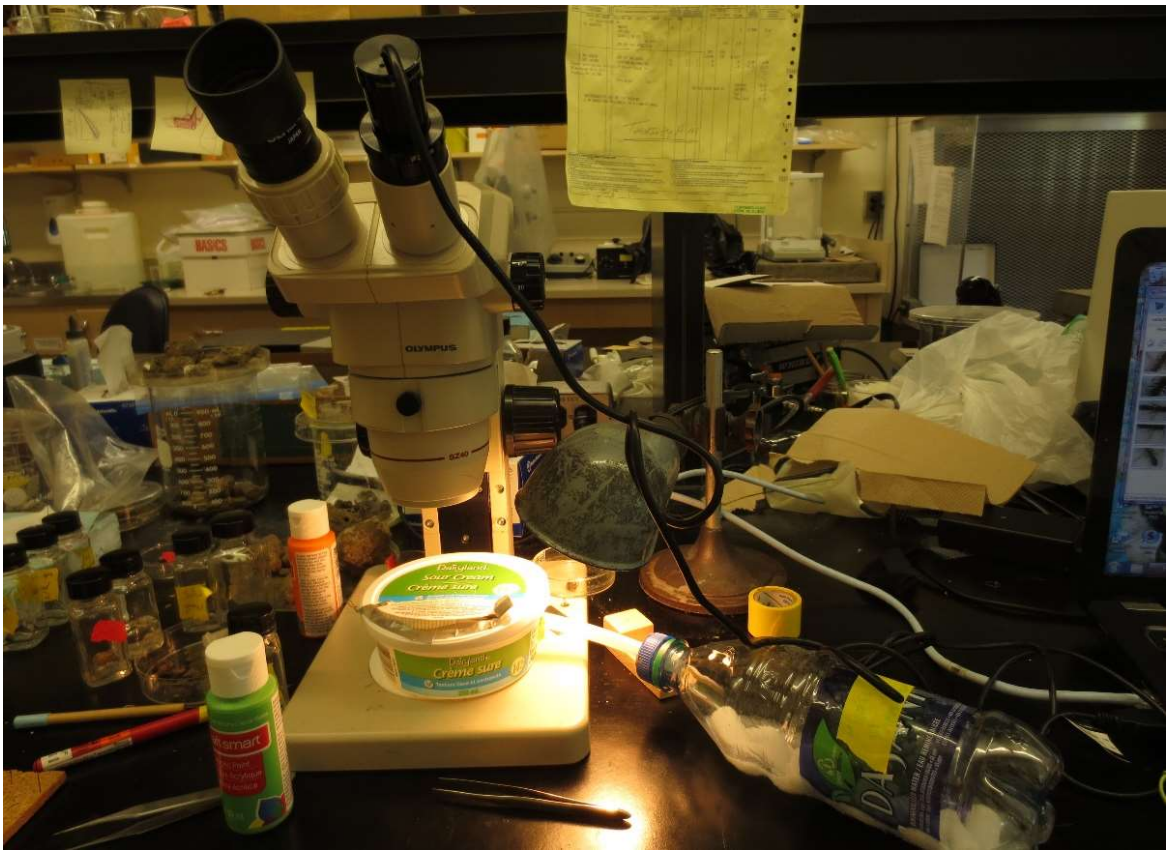


Fig 3.2. Set-up used to anaesthetize adult *Halictus rubicundus* using CO_2. Each bee was sealed in the flat plastic dish resting on the microscope stage. A small window in the dish lid permitted observation of cessation of bee movement, owing to CO_2 entrance to the dish via PVC tubing which connected the horizontal water bottle at the right. Dry ice in the bottle served as the CO_2 source. A Dinocapture camera was inserted in place of the right ocular and connected to the laptop computer to the right of this picture.

wedged into the bottle cap and sealed with glue. A bee was placed in the holding chamber and photographed when it stopped moving. Original plans involved the use of non-toxic paint applied to the mite dorsum to identify individual deutonymphs, but this method was found to be inefficient. Given the limited amount of time the bee could spend anaesthetized, there was not enough time to paint each deutonymph. In addition, the smallest possible brush (an eye lash hair) was still too large, prone to clinging and the paint dried too quickly. Instead, photographs were taken before and after the treatment period, which allowed the two images to be compared in order to detect any mite movements.

3.2.5.3 Collection of deceased bees

As part of an effort to determine whether mites uniformly deserted their hosts upon death, dead *Halictus rubicundus* were collected from site 1 and viewed under a dissecting microscope when return to the lab. These bees were deceased for an unknown period of time before collection.

3.2.6 Comparison to other mites found on halictids and local bees

Throughout the field seasons other insects, with a bias towards foraging bees, were collected in addition to *Halictus rubicundus,* and observed under a dissecting microscope for the presence or absence of mites. These insects were collected either as a part of the preliminary project (2013), or during the early stages of the graduate project (Appendix 2,3).

3.3 Results

3.3.1 Mite distribution on adult bees

Mites were typically directly and securely attached to a bee body's external surface, as opposed to loosely caught in the hairs on an adult bee. Upon examination of adult bees in the laboratory, only 3 (0.0895%) of the 3,351 mites (all deutonymphs) on adult bees of *H. rubicundus* were loosely attached, and they are included in the data below. Bees were divided into separate groups based on approximate age (i.e., larva, pupa, pharate, adult), as well as sex (i.e., post-larval stages). Table 3.1 summarizes the mite loads per bee life stage and sex, as elaborated below.

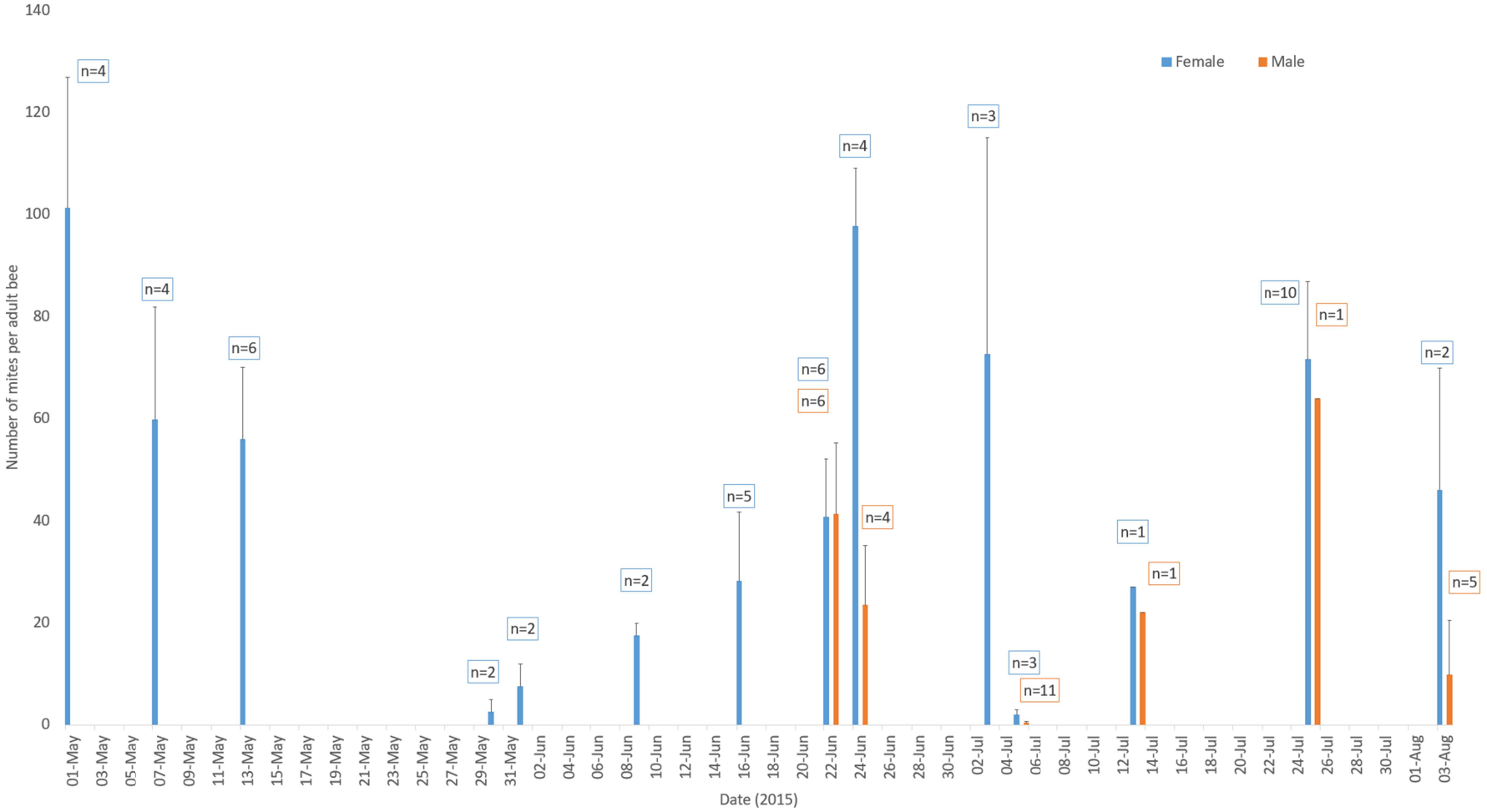


Fig 3.3. Average number of *Anoetus halictonida* mites per body of adult male and female bees of *Halictus rubicundus* collected during excavations at site 1 during spring-summer of 2015. Standard error bars are accompanied by the total number of adult bees (n) collected on each particular date.

As shown in Fig 3.3, the average numbers of mites on adult bees showed no obvious trend. Both male and female data showed peaks throughout the field season, accompanied by very deep troughs. Average mite loads of males and females were highly variable throughout the field season and among groups collected on the same day

Among adult female bees, the total number of attached mites ranged from 0 to 167, with a grand total of 2,870 mites found on the 54 adult female bees examined. Of the latter, two (one on May 30, 2015, the other on July 3, 2015) had no mites (3.7%), and of those, one bee had been attacked by a parasitoid. The average number of mites was high in early May and late June and July 2, 2015, but also had several dramatic lows in late May and on 4 July 2015 (Fig 3.4). The average number of total mites per body for all 54 female bees was 53.1 ± 44.6 (s.d.). There were several adult female bees (25.9%) with mite loads up to 20 or fewer per bee (Fig 3.4). However, almost half of the bees had mite loads of 21-80 mites per bee, and the trend showed a gradual decline in mite frequency per bee as mite load increased (Fig 3.4).

The total number of mites on adult male bees ranged from 0 to 77, with a grand total of 481 mites found on the 28 adult male bees examined. Of the total adult male bees, 12 had no mites (42.9%). The average number of total mites per body for all 28 collected adult males was 17.2 ± 25.6 (s.d.). Of the 28 male bees illustrated in Fig 3.5, a large proportion (64.3%) had mite loads of 10 mites or less per bee (Fig 3.5). Any mite load above this low level was relatively uncommon (Fig 3.5).

Adult male bees had lower overall numbers of mites, a more limited range, and a much higher proportion had no mites at all when compared to adult female bees (Fig 3.3-3.5, Table 3.1). There were fewer male adult bees collected in total, necessitating some caution when comparing the two. The total number of adult female bees (n=54) taken during nest excavations at site 1, was almost double (1.93-fold) that of male bees (n=28). However, there were nearly six times (5.97-fold) the number of mites collected on adult female bees (2,870 mites) as compared to male bees (481 mites), with 96.3% of adult females, but only 57.1% of adult males, hosting any mites.

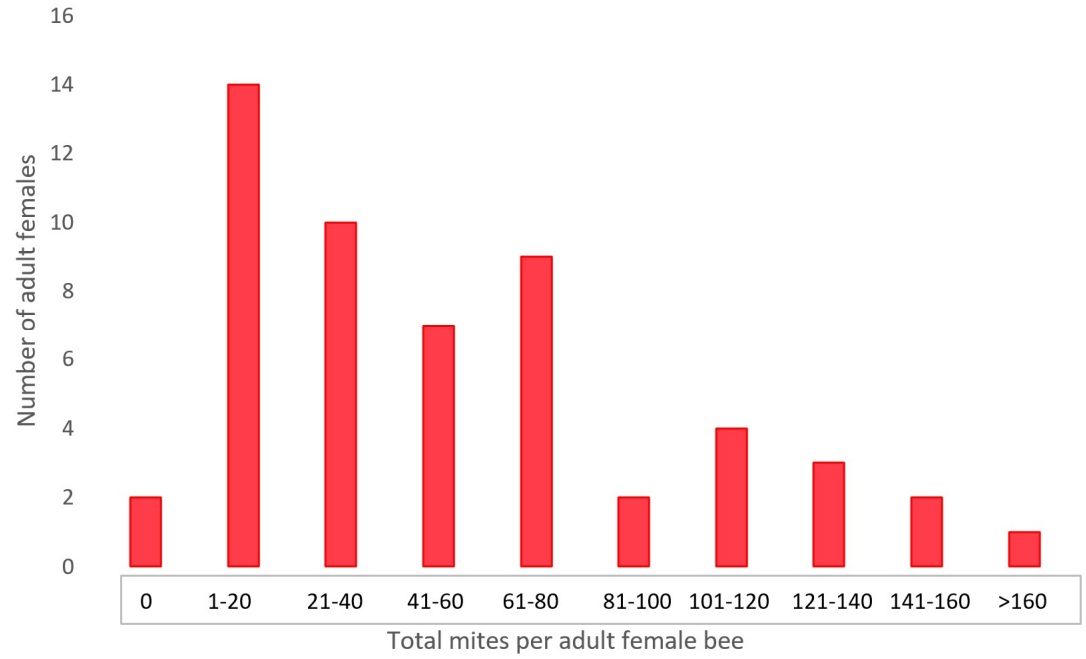


Fig 3.4. Frequency of the total number of mites of *Anoetus halictonida* per adult female bee of *Halictus rubicundus* collected during excavation at site 1 during the spring and summer of the 2015 field season.

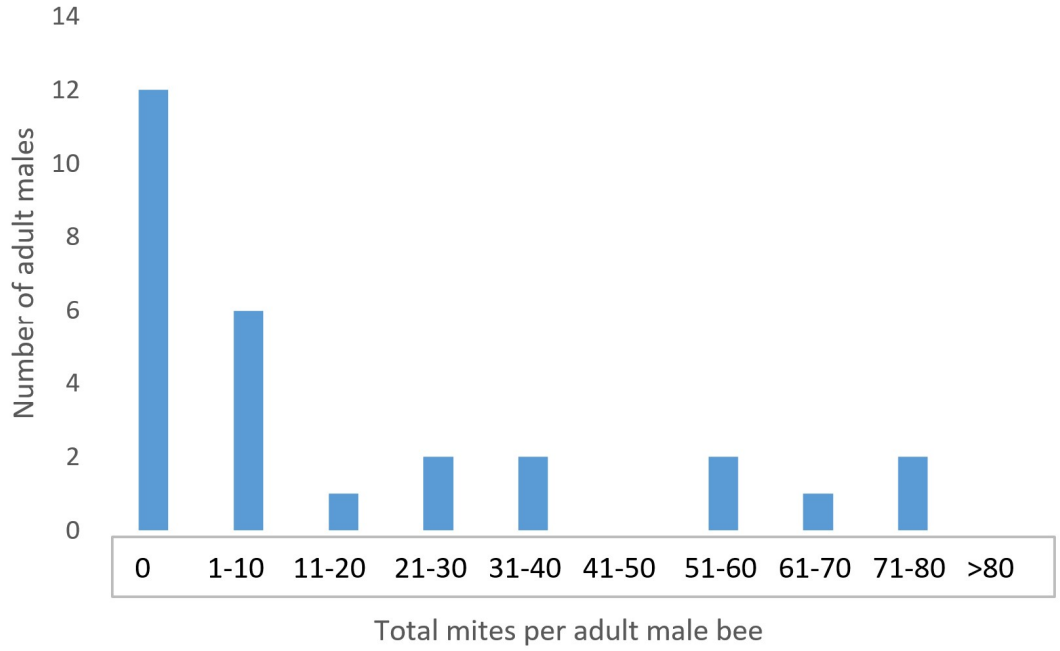


Fig 3.5. Frequency of the total number of mites of *Anoetus halictonida* per adult male bee of *Halictus rubicundus* collected from excavated nests at site 1 throughout 2015.

3.3.2 Mite distribution on immature bees

Certain life stages, namely the pupae and the pharate individuals, while technically separate based on age were comparable in mite distribution on the immature bee body. Mites were often easier to remove, if somewhat harder to access because of the compact nature of the appendages (i.e., legs tucked in and wings folded over) of the exarate pupae of *Halictus rubicundus*. Mites were not directly attached to the surface of the immature pupae and pharate pupae, instead being located on the exuvium which separates them from the immatures. Mites on immatures were of varying stages, although it is believed most were deutonymphs with the rarer pharate tritonymph harder to distinguish. The presence of multiple life stages of mites on immature bees is markedly different when compared to the monopoly that deutonymphs seemed to display on adults.

The total number of mites attached to immature female bees (specifically all stages of pupae and the phenologically mature pharate individuals) ranged from 0 to 134, with a grand total of 1,341 mites found on the 40 immature female bees. Mites were found on immature female bees excavated from June 9-August 3, 2015 (Fig 3.6). Of the 30 pupae, two (6.7%) had no mites (Fig 3.7). All of the female pharate individuals (n=10) had some mites (Fig 3.8). The average total number of mites per body for all 40 immature female bees was 32.6 ± 31.0 (s.d.).

The frequency distribution of mite load per female pupa (Fig 3.7) showed that half of female pupae have mite loads below 20, although larger loads were not uncommon. Of the total pupae (n=30) illustrated in Fig 3.7, the average mite load was 30.9 ± 29.0 (s.d.), the range was 0-111, and the total mites tallied 927. As illustrated in Fig 3.8, many of the pharate female individuals have mite loads of less than 40. For the individuals (n=10) represented in Fig 3.8, the average mite load was 41.4 ± 37.6 (s.d.), the range was 6-134, and the total mites tallied 414. Among immature male bees, the total number of attached mites ranged from 0 to 116, with a grand total of 790 mites found on the 22 post-larval males collected from June 9 – July 13, 2017 (Fig 3.6). Of these individuals, three (one pharate stage and two pupae) had no mites (13.6%). The average total number of mites per body for all 22 pupae and pharate-staged males was 35.9 ± 41.3 (s.d.).

The frequency distribution of mite load per male pupa (Fig 3.9) illustrates that the majority had mite loads below 40, whereas very few have either none or >100. Of the male pupae

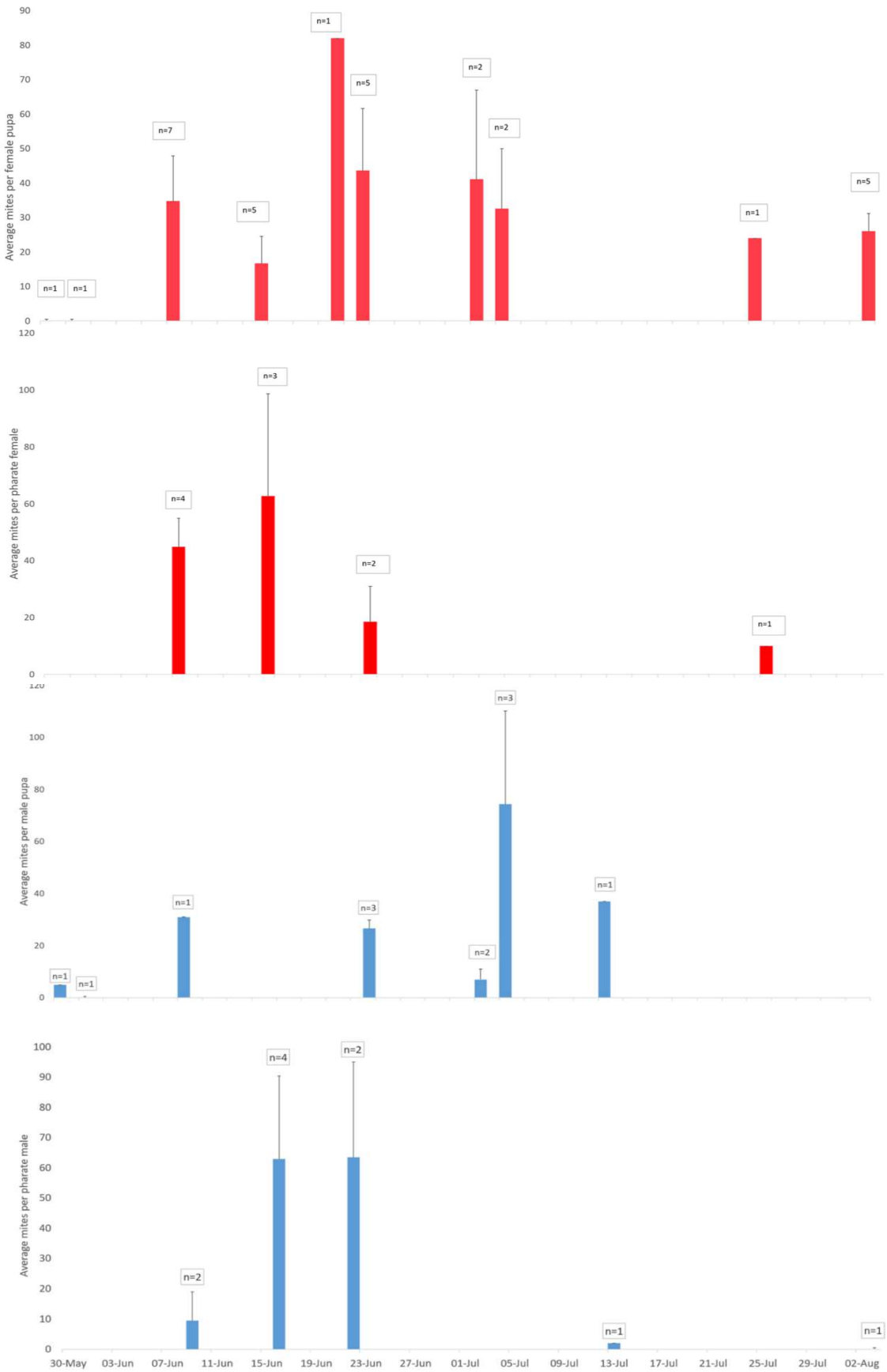

Average mites per female pupa
Average mites per pharate female
Average mites per male pupa
Average mites per pharate male
n=1
n=7
n=5
n=2
n=4
n=3
30-May
03-Jun
07-Jun
11-Jun
15-Jun
19-Jun
23-Jun
27-Jun
01-Jul
05-Jul
09-Jul
13-Jul
17-Jul
21-Jul
25-Jul
29-Jul
02-Aug

Fig 3.6. Average number of mites of *Anoetus halictonida* per body of female pupae, female pharate individuals, male pupae, and male pharate individuals of *Halictus rubicundus* collected during excavations at site 1 during spring-summer of 2015. Standard error bars are accompanied by the total number of bees (n) on date of collection.

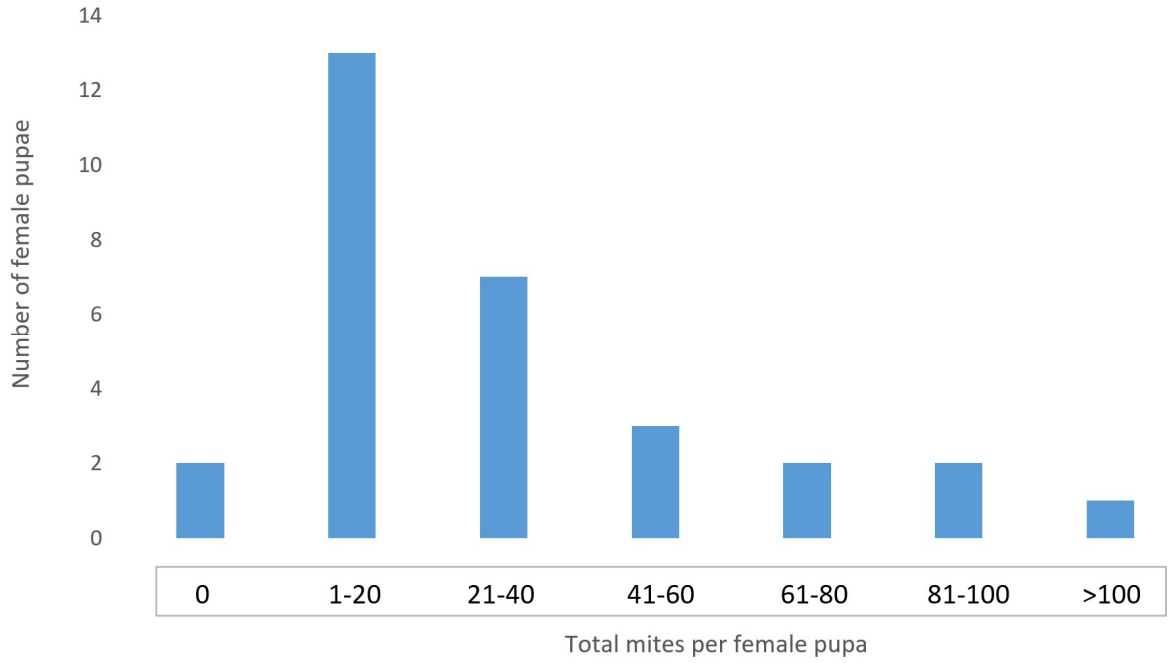


Fig 3.7. Frequency of mites of *Anoetus halictonida* per female pupa of *Halictus rubicundus* (n=30) excavated from site 1 during 2015.

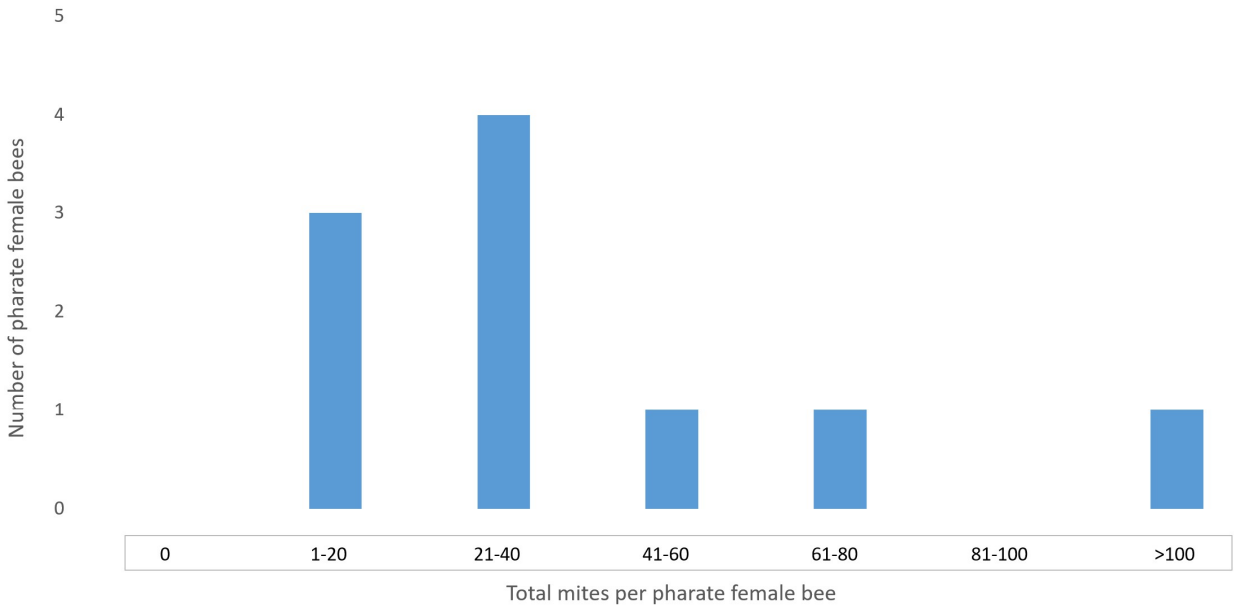


Fig 3.8. Frequency of mites of *Anoetus halictonida* per pharate female individual of *Halictus rubicundus* (n=10) excavated from site 1 during 2015.

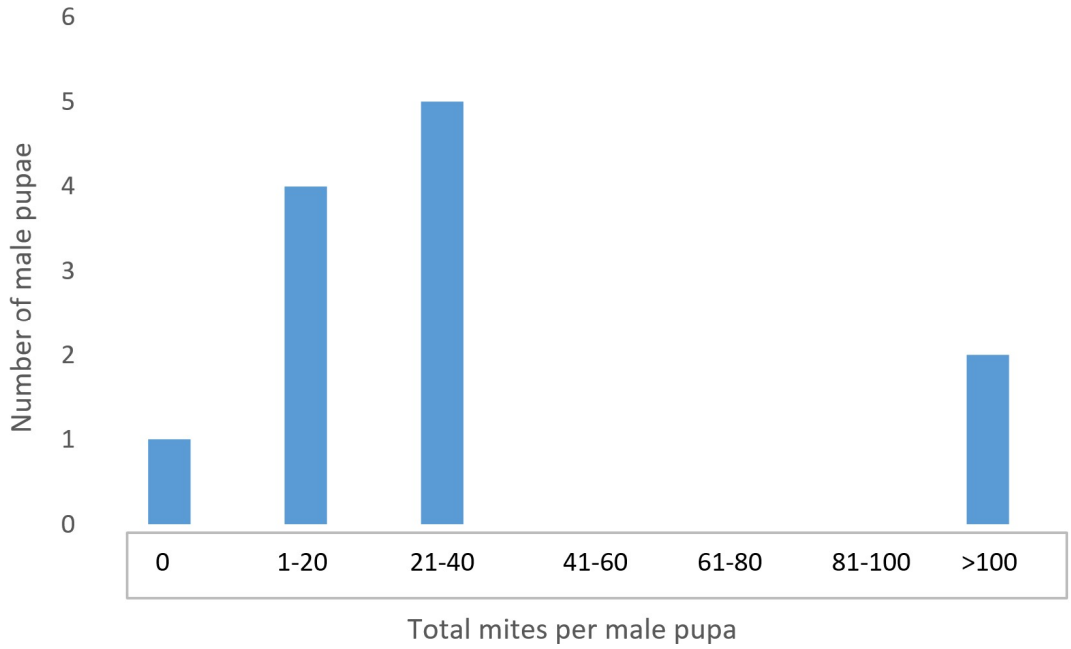


Fig 3.9. Frequency of mites of *Anoetus halictonida* per male pupa of *Halictus rubicundus* (n=12) from site 1 during 2015.

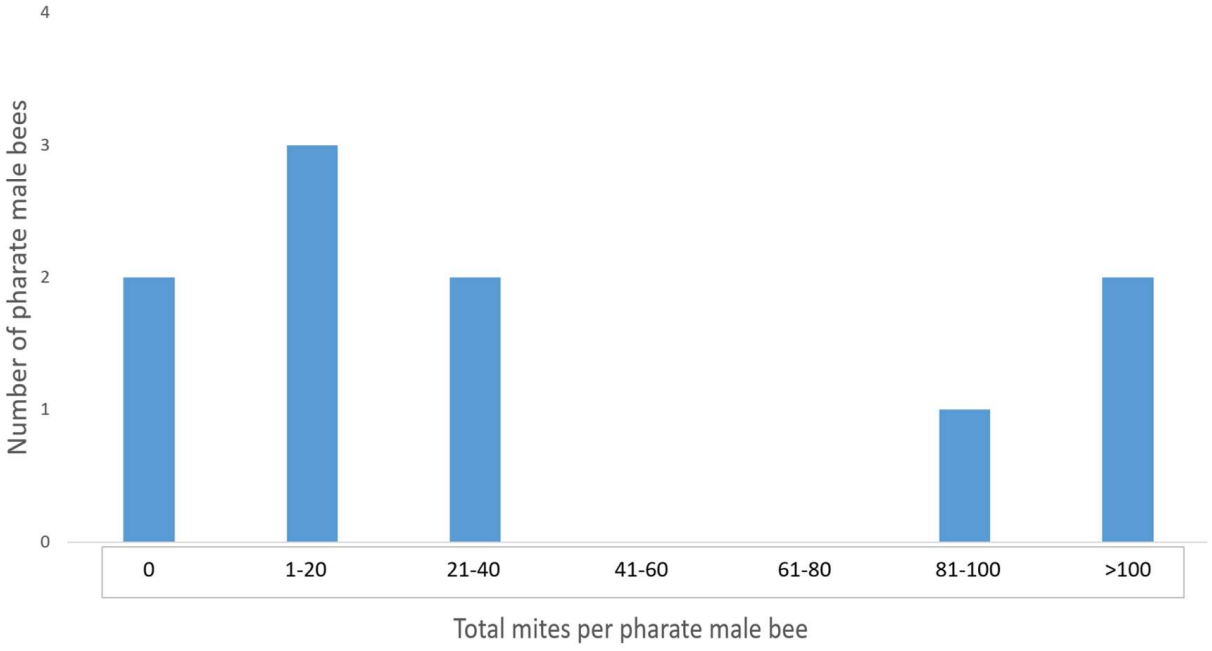


Fig 3.10. Frequency of mites of *Anoetus halictonida* per male pharate individual (n=10) of *Halictus rubicundus* from site 1 during the 2015 field season.

graphed (n=12), the average mite load was 32.5 ± 38.5 (s.d.), the range was 0-115, and the total mites tallied 390. From Fig 3.10, it is evident that several male bees at the pharate stage have either less than 40 mites on their body, or greater than 80 mites. Of the pharate males graphed (n=10), the average mite load was 40.0 ± 46.3 (s.d.), the range was 0-116, and the total mites tallied 400. There were nearly equal numbers of male pupae and pharate individuals, and the two groups had similar total numbers of mites and comparable trends. That is, the majority of male immatures bees have a mite load that is either very low or very high, with a lack of representatives between the two extremes.

The trend when viewing female pupae and pharate individuals is a much more gradual decline, with many of the females having mite loads of 1-40. Of the female pupae (n=30), 20 (66.7%) fit this description, whereas among the pharate females (n=10), 7 (70%) individuals fell within this category. Of the male pupae, 9 of the 12 (75%) had mite loads between 1-40, and 5 of 10 (50%) pharate males have mite loads from 1-40.

Table 3.1 Summary of average loads of mites of *Anoetus halictonida* for post-larval male and female bees of *Halictus rubicundus* recovered during nest excavation at site 1 in 2015.

Female	**n**	**Mean ± s.d.**		**Male**	**n**	**Mean ± s.d.**
Adult	54	53.1 ± 44.6		Adult	28	17.2 ± 25.6
Immature	40	32.6 ± 31.0		Immature	22	35.9 ± 41.3
Pharate	10	41.4 ± 37.6		Pharate	10	40.0 ± 46.3
Pupae	30	30.9 ± 29.0		Pupae	12	32.5 ± 38.5

3.3.3 Localized mite distribution on bees

3.3.3.1 Mite distribution on adult female bees

Deutonymphs of *Anoetus halictonida* were the only life stage found on adult female bees of *Halictus rubicundus*. The body regions with high frequencies of mites were the propodeum, the first abdominal tergite, and the thoracic regions adjacent to the wing bases (Fig 3.11, 3.19a). No mites were found on the distal leg segments (i.e., tibia, tarsus), the antennae, or elsewhere on the head. Sternite 1, which is spatially close to the propodeum and tergite 1 had the highest mite frequency of any of the ventral abdominal body regions. Those mites on sternite 1 tended towards

the middle of the sternite, away from the basal leg segments, and were numerically restricted as compared to those mites on tergite 1. Instead of occupying the anterior or posterior regions of the adult female host, most mites not on wings resided near the junction of the thorax and abdomen. Deutonymphs predominated on the lower surfaces of the wings (76.08%; Fig 3.19b), especially around the main veins (Fig 3.12). The upper surfaces of the wings were less populated (3.96%), but mites were occasionally present of the jugal lobe of the hind wing (Fig 3.12), which had a tendency to fold over when the bee folded its wings.

For adult female bees of *H. rubicundus*, 80.04% of deutonymphs of *A. halictonida* occupied the wings as shown in Fig 3.12, whereas 20.15% of deutonymphs resided on the thorax and abdomen as illustrated in Fig 3.11, yielding a 4-fold increase of mites on the wings.

Dividing the host body into regions allowed investigation into whether the mite distributions were symmetrical (i.e., the null hypothesis), or if they were significantly asymmetrical. The forewings and hindwings of *H. rubicundus* only appeared to have a left-right dissymmetry when mite loads on adult female bees were analysed as individuals. Out of the total adult female *H. rubicundus*, 8 had no mites on their forewings. Of the remaining 46 bees, only 8 (17.4%) had a significant favouring of one side ($P<0.05$), with 5 bees hosting significantly more deutonymphs on their left forewings, and 3 bees with higher mite loads on their right forewings. The most asymmetrical examples appeared later in the year on July 3, 2015 (24L:6R) and on August 3, 2015 (2L:15R).

Of the total 54 adult female *H. rubicundus*, 4 had no mites on their hindwings. Of the remaining 50, 11 (22.0%) had a significant left-right dissimilarity ($P<0.05$), with 8 bees having significantly more deutonymphs on their left hindwings, and 3 bees with higher mite loads on their right hindwings. The most asymmetrical examples were females from separate nests on July 25, 2015, both showing a significant asymmetrical distribution favouring the left hindwing (22L:8R, 26L:6R).

When the total number of deutonymphs of *A. halictonida* residing on all four regions of interest (forewing-left, forewing-right, hindwing-left, hindwing-right) were combined within a 2 X 2 Chi-square table (Table 2.3), the summed Chi-square value (0.8234) did not exceed the critical Chi-square value of 3.84 ($P=0.05$, 1 degree of freedom). Therefore, the overall

distribution of mites on wings for this population of adult bees of *H. rubicundus* appeared symmetrical, and hence the null hypothesis was accepted (P> 0.30).

Despite this symmetrical arrangment for deutonymphs of *A. halictonida* on the wings, a separate chi-square analysis demonstrated that the 1,324 mites residing on the hindwings (versus 974 mites on the forewings; see Table 3.2) represented a highly significant difference (chi-square value= 53.30, P<0.0005). Therefore, even though hindwings have a smaller surface than forewings – a factor not incorporated into this analysis – the hindwings of adult female bees of *H. rubicundus* were significantly more populated by deutonymphs of *A. halictonida* than the forewings.

Table 3.2 Analysis of left-right symmetry of overall distribution of deutonymphs of *Anoetus halictonida* on wings of adult female bees of *Halictus rubicundus* captured during nest excavations at site 1 throughout spring-summer 2015, using a 2x2 chi-square table. Mite load data on bee wings represent 52 adult female bees.

	Forewing	Hindwing	Total
Left side	500 (Observed) Expected= (AxC)/E =510.735 Chi-square= 0.2257	705 (Observed) Expected= (BxC)/E =694.265 Chi-square= 0.1660	1,205 (=C)
Right side	474 (Observed) Expected= (AxD)/E =463.265 Chi-square= 0.2488	619 (Observed) Expected= (BxD)/E =629.735 Chi-square= 0.1830	1,093 (=D)
Total	974 (=A)	1,324 (=B)	2,298 (=E)

3.3.3.2 Mite distribution on adult male bees

Deutonymphs were the only mite stage found on adult male bees of *Halictus rubicundus*. The deutonymphs were often found on the ventral body regions (Fig 3.13), with the basal leg segments (Fig 3.21c), such as the coxae, the areas between the coxae (Fig 3.20a,b), and the underside of the head near the mouthparts (Fig 3.21a,b, 3.25b) all having high proportions of

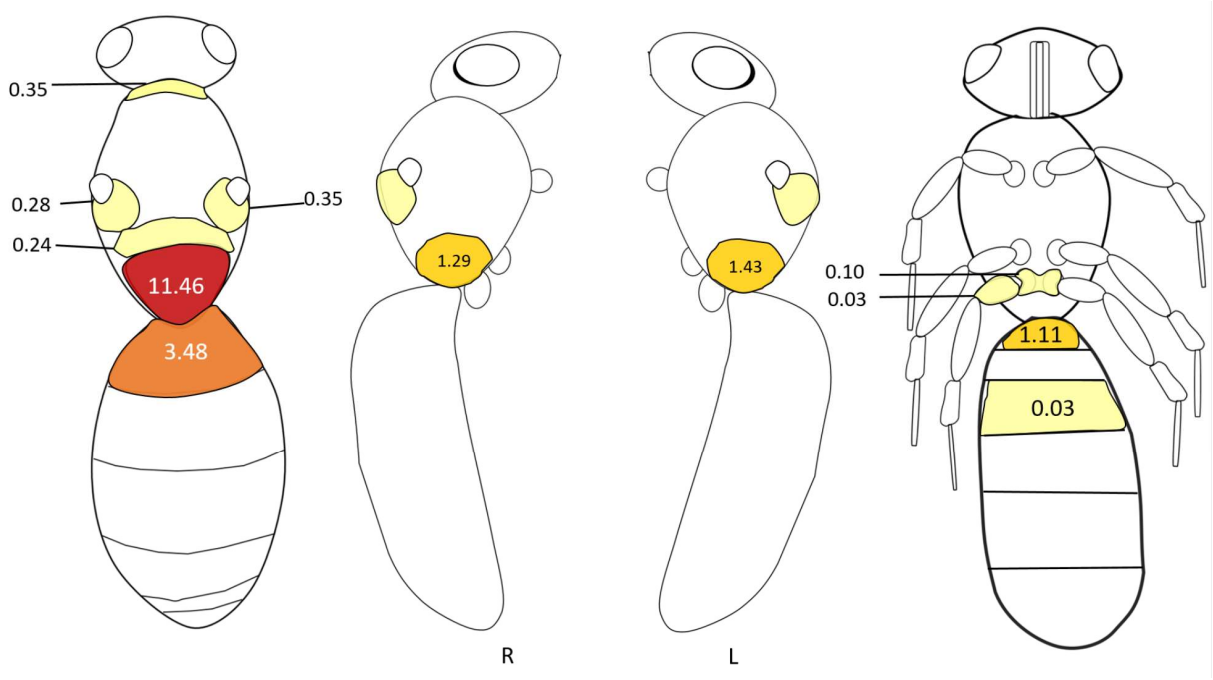


Fig 3.11. Dorsal, lateral (right, R; left, L), and ventral representations showing proportional distribution of deutonymphs of *Anoetus halictonida* on adult female *Halictus rubicundus* collected during nest excavations at site 1 in 2015. Diagrams are limited to the three body domains (head, thorax, abdomen) of the adult bee, excluding the wings (see Fig 3.12). Darker shading indicates a higher percentage of mites, whereas white denotes no mites found (0.01-0.49%, 0.50-0.99%, 1.0-1.99%, 2.0-3.99%, 4.0-7.99%, 8.0-15.99%).

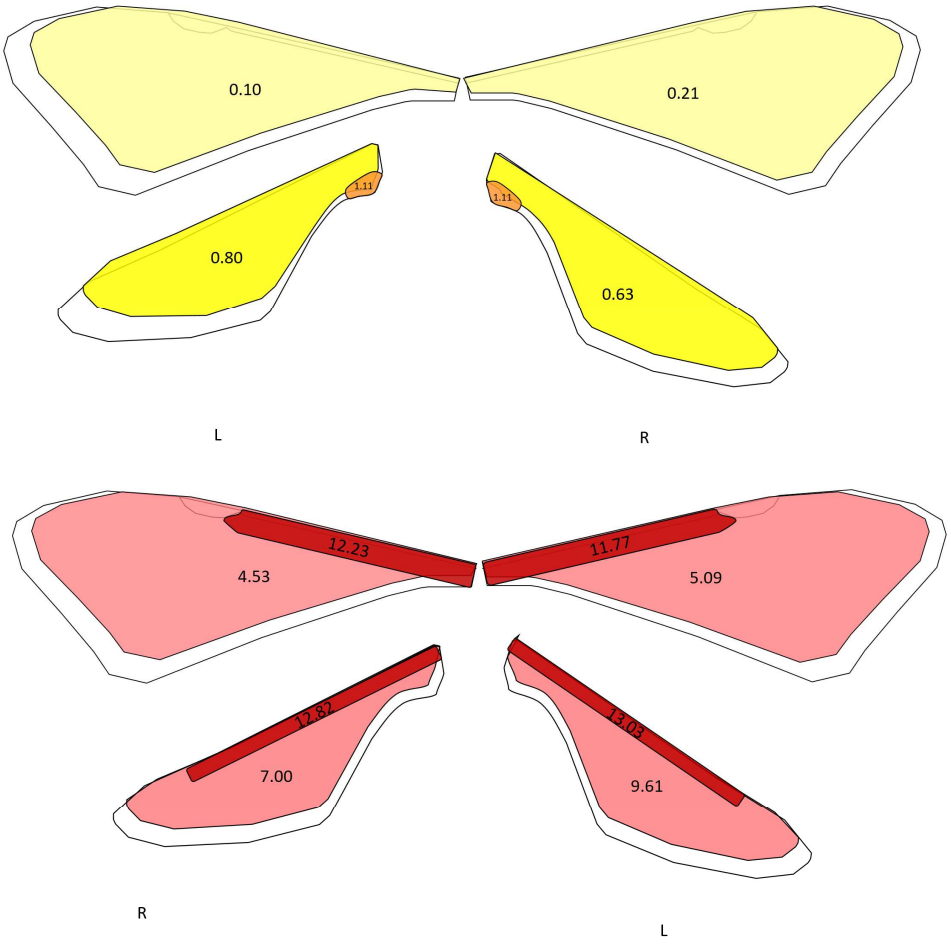


Fig 3.12. Schematic representations of the proportional distribution of deutonymphs of *Anoetus halictonida* on the upper surface (above) and lower surface (below) of the wings (right, R; left, L) of adult female *Halictus rubicundus* collected during nest excavations at site 1 in 2015. Hind wings have jugal lobes indicated. For the lower wing surfaces, the darkened rectangular areas illustrate the wing margins and comprise the main vein regions of the fore and hind wings. Darker shading indicates a higher percentage of mites, whereas white denotes no mites found (0.01-0.49%, 0.50-0.99%, 1.0-1.99%, 2.0-3.99%, 4.0-7.99%, 8.0-15.99%).

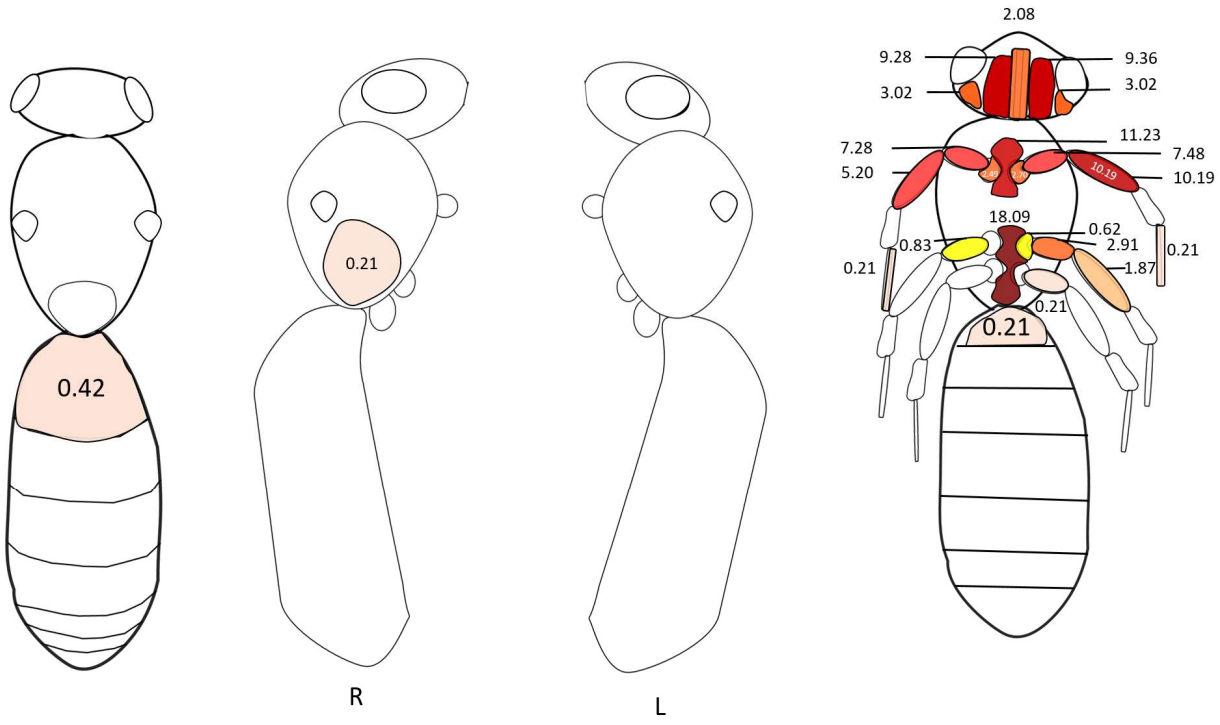


Fig 3.13. Dorsal, lateral (right, R; left, L), and ventral representations showing proportional distribution of deutonymphs of *Anoetus halictonida* on adult male *Halictus rubicundus* collected during nest excavations at site 1 in 2015. Diagrams are limited to the three body domains (head, thorax, abdomen) of the adult male bee, excluding the wings (see Fig 3.14). Darker shading indicates a higher percentage of mites, whereas white denotes no mites found (0.01-0.49%, 0.50-0.99%, 1.0-1.99%, 2.0-3.99%, 4.0-7.99%, 8.0-15.99%).

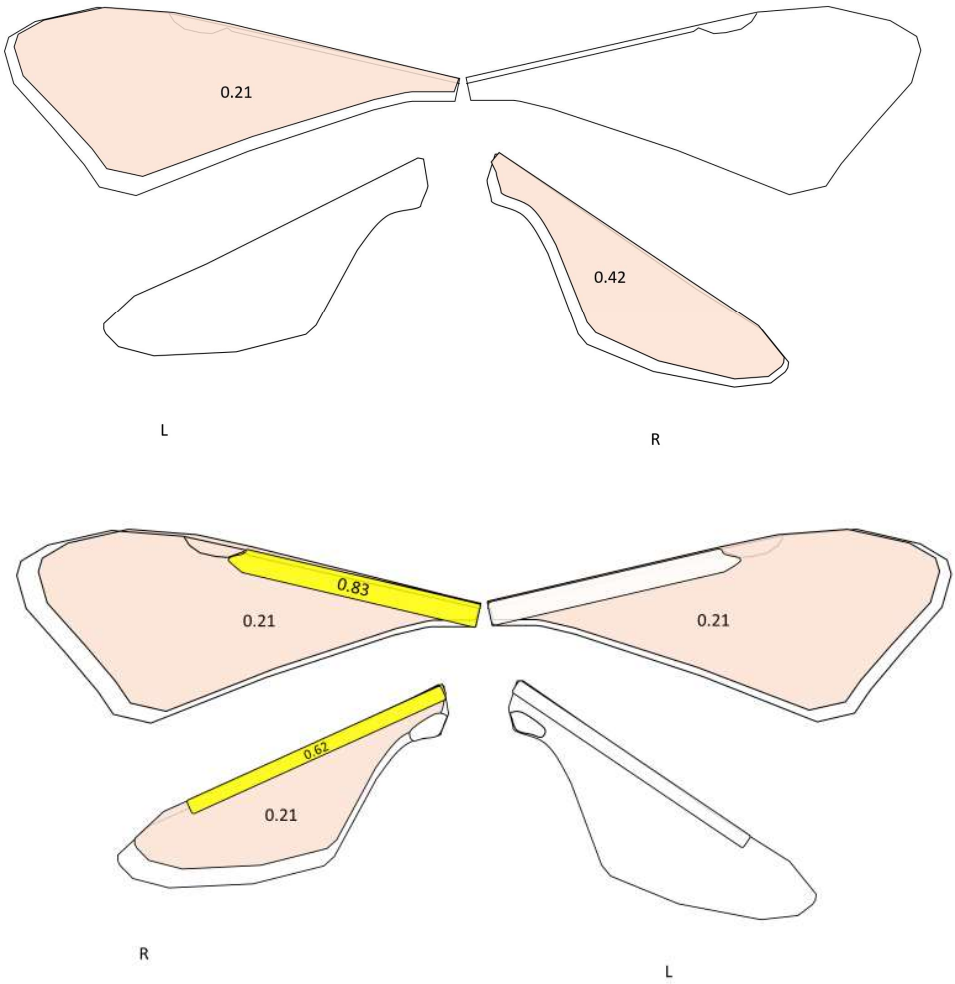


Fig 3.14. Schematic representations of the proportional distribution of deutonymphs of *Anoetus halictonida* on the upper surface (above) and lower surface (below) of the wings (right, R; left, L) of adult male *Halictus rubicundus* collected during nest excavations at site 1 in 2015. The lower wings have the jugal lobes and main vein areas as separate regions from the rest of the wings. Darker shading indicates a higher percentage of mites, whereas white indicates no mites found (0.01-0.49%, 0.50-0.99%, 1.0-1.99%, 2.0-3.99%, 4.0-7.99%, 8.0-15.99%).

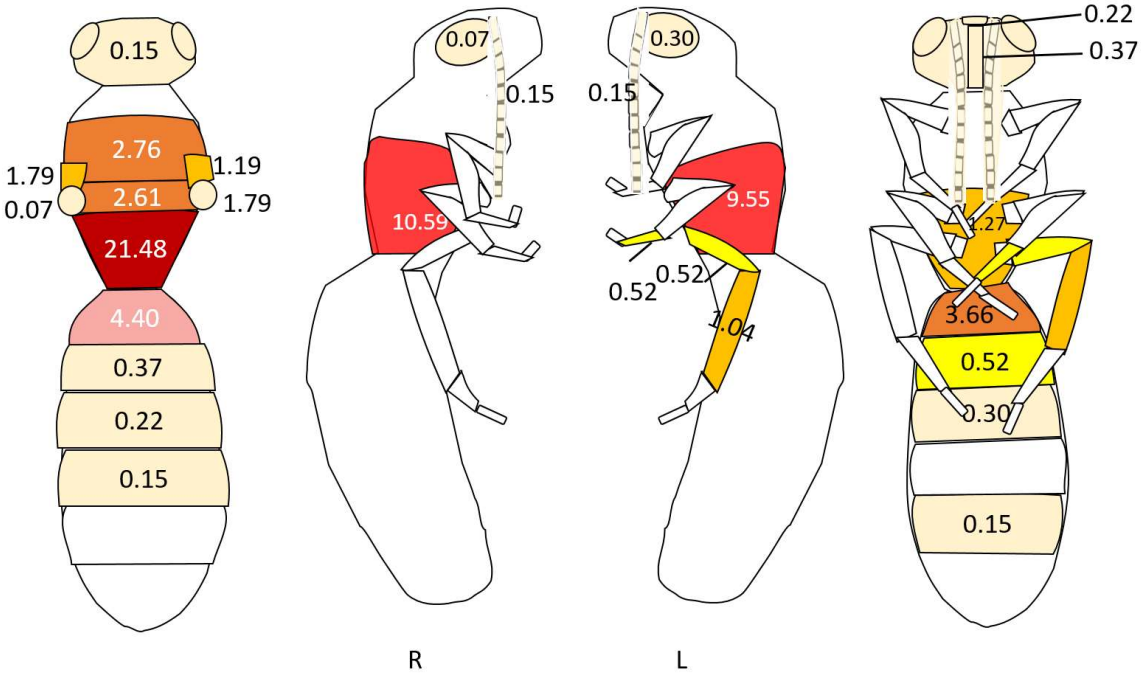


Fig 3.15. Dorsal, lateral (right, R; left, L), and ventral representations showing proportional distribution of *Anoetus halictonida* combined on post-larval immature stages (pupae and pharate individuals) of female *Halictus rubicundus* (n=40) collected during nest excavations at site 1 in 2015. Diagrams represent the three body domains (head, thorax, abdomen) of the immature bee, excluding the wings (see Fig 3.16). Darker shading indicates a higher percentage of mites, whereas white denotes no mites found (0.01-0.49%, 0.50-0.99%, 1.0-1.99%, 2.0-3.99%, 4.0-7.99%, 8.0-15.99%). Mites distributed on leg segments are shown in lateral (R,L) view, except for those on coxae which are illustrated in ventral view. For ease of reference, shading on leg segments in ventral view is retained.

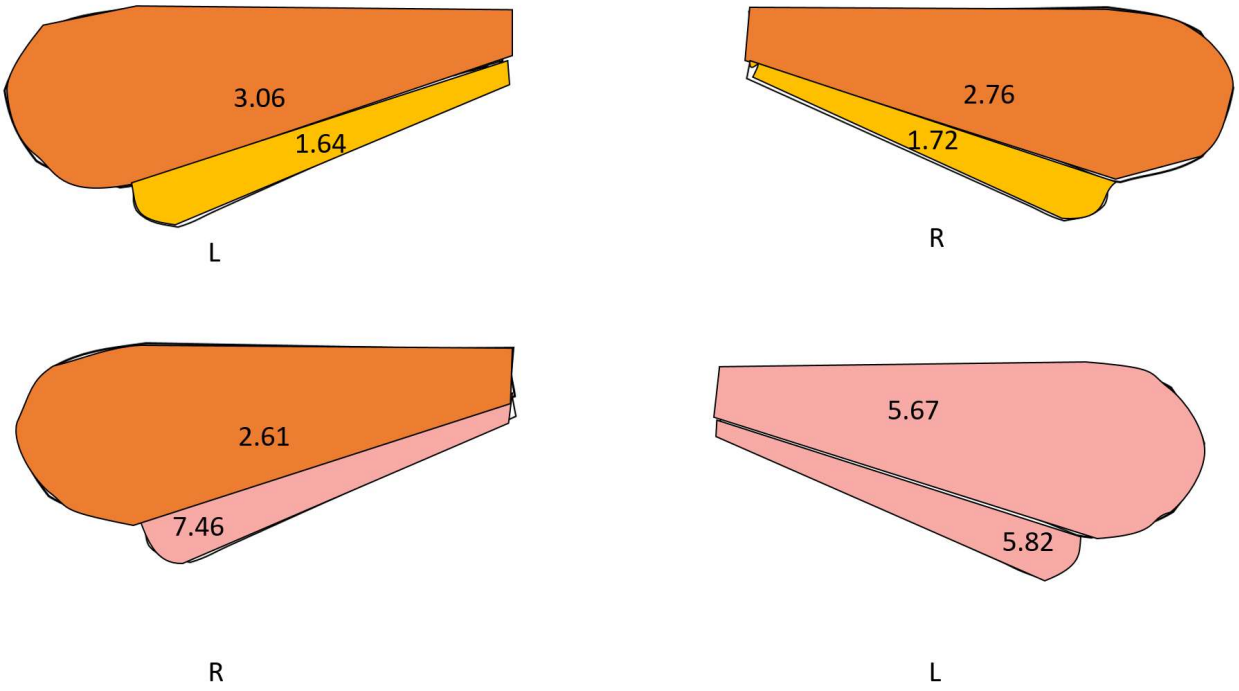


Fig 3.16. Proportional distribution of deutonymphs of *Anoetus halictonida* on the upper surface (above) and lower surface (below) of the wings on combined post-larval immature stages (pupae and pharate individuals) of female *Halictus rubicundus* (n=40) collected during nest excavations at site 1. Darker shading indicates a higher percentage of mites, whereas white denotes no mites found (0.01-0.49%, 0.50-0.99%, 1.0-1.99%, 2.0-3.99%, 4.0-7.99%, 8.0-15.99%).

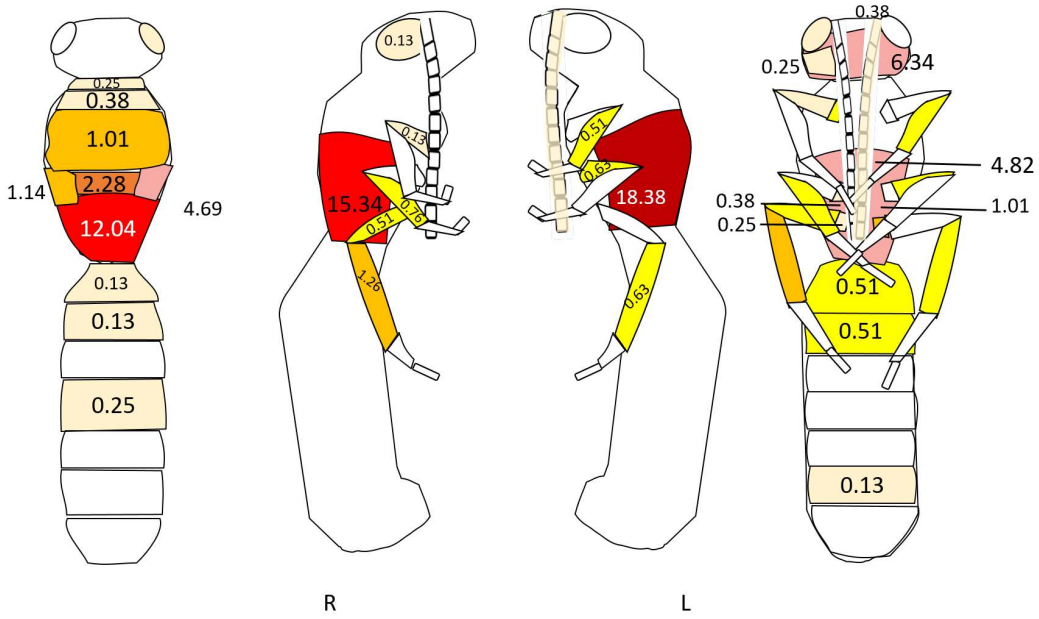


Fig 3.17. Dorsal, lateral (right, R; left, L), and ventral representations showing proportional distribution of *Anoetus halictonida* combined on post-larval immature stages (pupae, pharate individuals) of male *Halictus rubicundus* (n=22) collected during nest excavations at site 1 in 2015. Diagrams represent the three body domains (head, thorax, abdomen) of the immature bee, excluding the wings (see Fig 3.18). Darker shading indicates a higher percentage of mites, whereas white denotes no mites found (0.01-0.49%, 0.50-0.99%, 1.0-1.99%, 2.0-3.99%, 4.0-7.99%, 8.0-15.99%). Mites distributed on leg segments are shown in lateral (R,L) view, except for those on coxae which are illustrated in ventral view. For ease of reference, shading on leg segments in ventral view is retained.

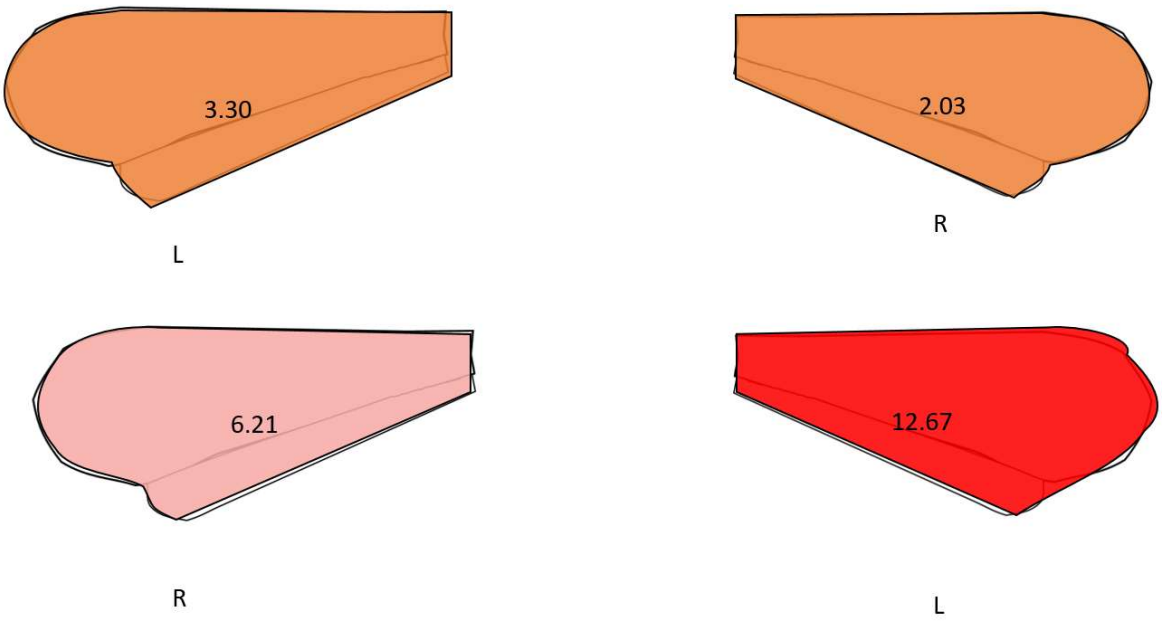


Fig 3.18. Proportional distribution of *Anoetus halictonida* on the upper surface (above) and lower surface (below) of the wings on combined post-larval immature stages (pupae, pharate individuals) of male *Halictus rubicundus* (n=22) illustrated in Fig 3.17, collected during nest excavations at site 1. Darker shading indicates a higher percentage of mites (0.01-0.49%, 0.50-0.99%, 1.0-1.99%, 2.0-3.99%, 4.0-7.99%, 8.0-15.99%).

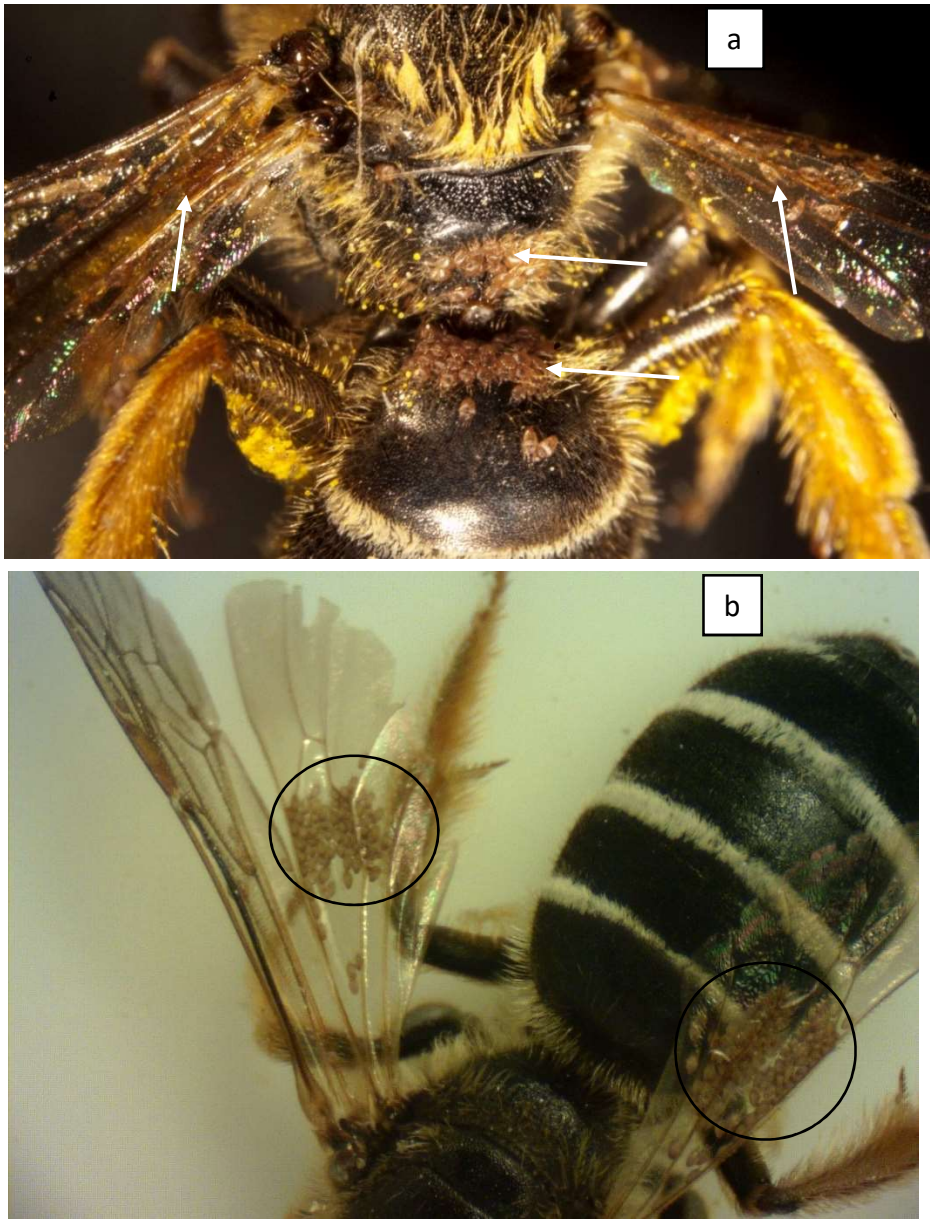


Fig 3.19. Deutonymphs of *Anoetus halictonida* on the body of adult female bees of *Halictus rubicundus*. a) Mites located on propodeum, wings, 1st tergite, and dorsal thorax of a pinned adult. Mites on propodeum and tergite 1 appear to oriented away from the mesosoma-metasomal junction. b) Mites located on the hindwings of an unpinned adult female. Mites appear oriented towards tip of wing.

Fig 3.20. Deutonymphs of *Anoetus halictonida* between the coxae of the mesothoracic legs on the ventral body surface of adult male bees of *Halictus rubicundus*. a) View of bee with dissecting microscope. b) Viewed with SEM.

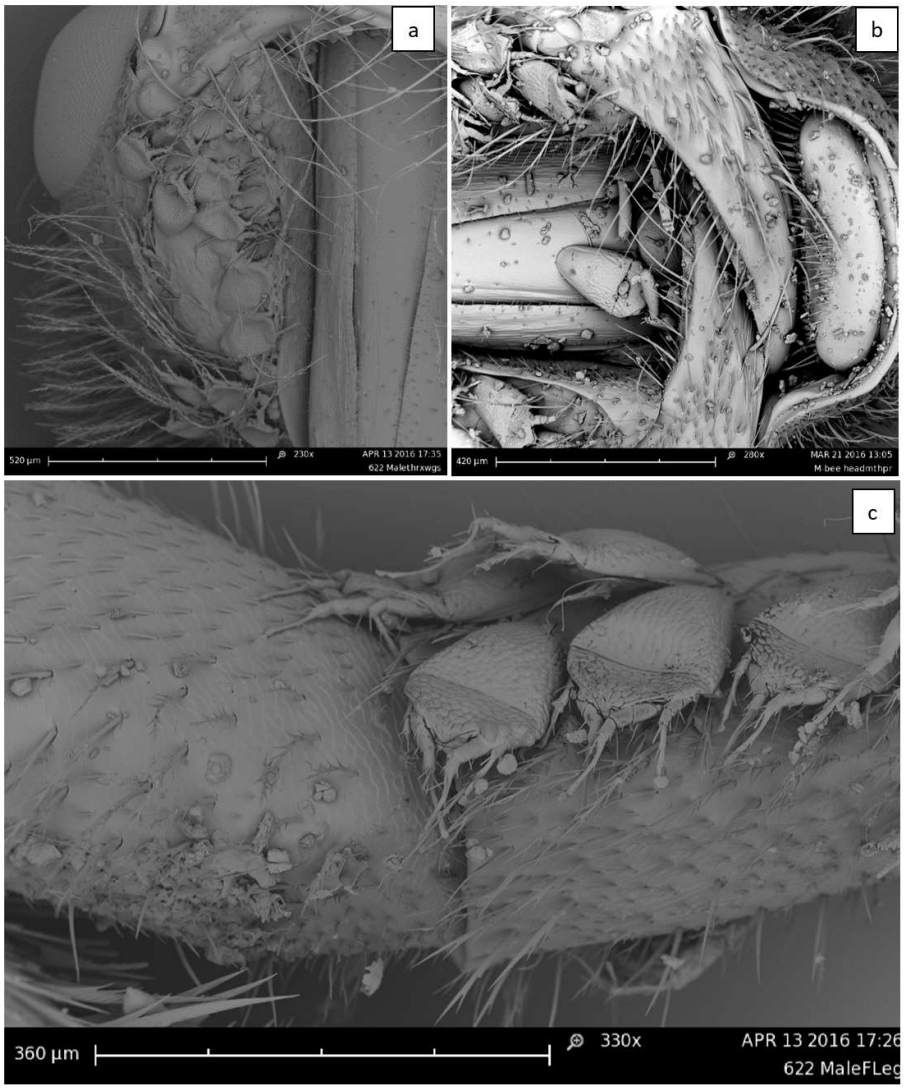


Fig 3.21 Deutonymphs of *Anoetus halictonida* on adult male bees of *Halictus rubicundus* illustrated by SEM. a) Mites attached beside proboscis in head region below right compound eye. b) Mites attached on mouthparts below mandibles. c) Mites stationed on right fore trochanter, near junction with femur.

mites. The mites were commonly positioned ventrally, but also towards the anterior end of the male bee (Fig 3.13). The mites on the leg segments were positioned closer to the head and were present in highest proportions on the fore leg segments (Fig 3.13, 3.21c). Adult male bees also had mites on their wings (Fig 3.14). Overall, the wings of adult male bees of *H. rubicundus* carried only 2.71% of the deutonymphs, the vast majority residing on other regions of the body (Fig 3.13).

Therefore, sex of the adult bee of *H. rubicundus* accounted for a major disparity in the localized distribution of *A. halictonida*, with 80% of the host body's deutonymphs occupying the wings in females, compared to less than 3% in males. Also, the former had a high proportion of their total mites especially near the main veins on the lower wing surfaces (Fig 3.12).

Whereas deutonymphs of *A. halictonida* most frequently resided on the dorsal surface of adult female bees and the ventral surface of the adult male bees, mite distribution in these regions was not absolute. Occasionally males had mites on their tergites (Fig 3.13 left), similar to females, and females occasionally had mites residing ventrally (Fig 3.11 right). However, this reversal was uncommon and hence proportions of total deutonymphs in these regions of the opposite sex were very low (<1.5% in Fig 3.11; <0.5% in Fig 3.13 left).

3.3.3.3 Mite distribution on post-larval immature bees

As the mite distribution and the average mite loads (Table 3.1) between pupae and pharate individuals did not appear noticeably different, they are combined here and designated as post-larval stages. Post-larval immature females of *Halictus rubicundus* had relatively high numbers of mites of *Anoetus halictonida* located around the propodeum, first tergite, thorax, and wing surfaces (Fig 3.15 and 3.16). The distribution of mites was widespread, with few host body regions lacking mites (Fig 3.15). Many of the areas on the pupae and pharate bees shown in Fig 3.15 had very low numbers of mites, often only 0.05-1% of the total mites (n=1,341). Post-larval immature females, in comparison to adult females (Fig 3.11), had many areas occupied by at least a few mites, as opposed to a limited number of areas with relatively higher proportions of mites. Mites were present on post-larval females in body regions (i.e., leg segments, eyes, and more distal abdominal segments as seen in Fig 3.15) that lacked mites completely in adult female bees (Fig 3.11).

Unlike in adult females, there was little difference in proportions between the upper and lower wing surfaces of post-larval stages of the host (Fig 3.16), nor in the proportions (14.10% on the forewings, 16.64% on hindwings) on each wing pair (Fig 3.16). However, there were also some similarities between post-larval and adult females regarding which areas of their bodies had high proportions of mites, with both stages often having mites on the propodeum, first tergite, and lower wing surfaces. Pre-adult females have the majority of their mite load located on their thorax plus legs (58.09%), less than a third located on their wings (30.72%), a tenth on their abdomens (9.99%), and a fraction on their heads (1.42%). In comparison, adult female bees have the majority of their mites located on their wings (80.04%), with far fewer on their thoraces (15.53%) and abdomens (4.62%).

A grand total of 789 mites were collected from the post-larval stages (pupae, pharate individuals) from 22 males of *H. rubicundus*. The distribution of *A. halictonida* on post-larval immature males (Fig 3.17, 3.18) was not drastically different from that of the post-larval females (Fig 3.15, 3.16). Mites occupied many different areas including the upper and lower wing surfaces, the lateral, ventral and dorsal posterior areas of the thorax, and the leg segments (Fig 3.17, 3.18, 3.22a,b). The regions with the highest mite populations, in descending order, were the lateral left side of the thorax, lateral right side of the thorax, left wing lower surface, and the dorsal propodeum (Fig. 3.17, 3.18). Uncommonly populated areas included the eyes, antennae, and distal abdominal segments (Fig. 3.17). In comparison, adult males tended to have the majority of their mites on their venters (Fig 3.13), whereas post-larval males showed distributions of mites on their bodies more similar to female post-larval immatures. Post-larval males had the majority of their mites located on their thoraces plus legs (66.4%), about a quarter on their wings (24.21%), less than a tenth on their heads (7.10%), and relatively few on their abdomens (1.66%). These numbers are somewhat different from those of the post-larval females, i.e., the males show a much larger proportion of mites on their thoraces, but the relative order of body regions is the same. Adult male bees have the majority of mites located on their thoraces, and the proportion of mites (71.94%) is comparable to the proportion found on post-larval males in the same region. However, unlike in post-larval males, the body region with the next highest proportion of mites is the head (24.76%), then the wings (2.71%), and finally the abdomen (0.63%).

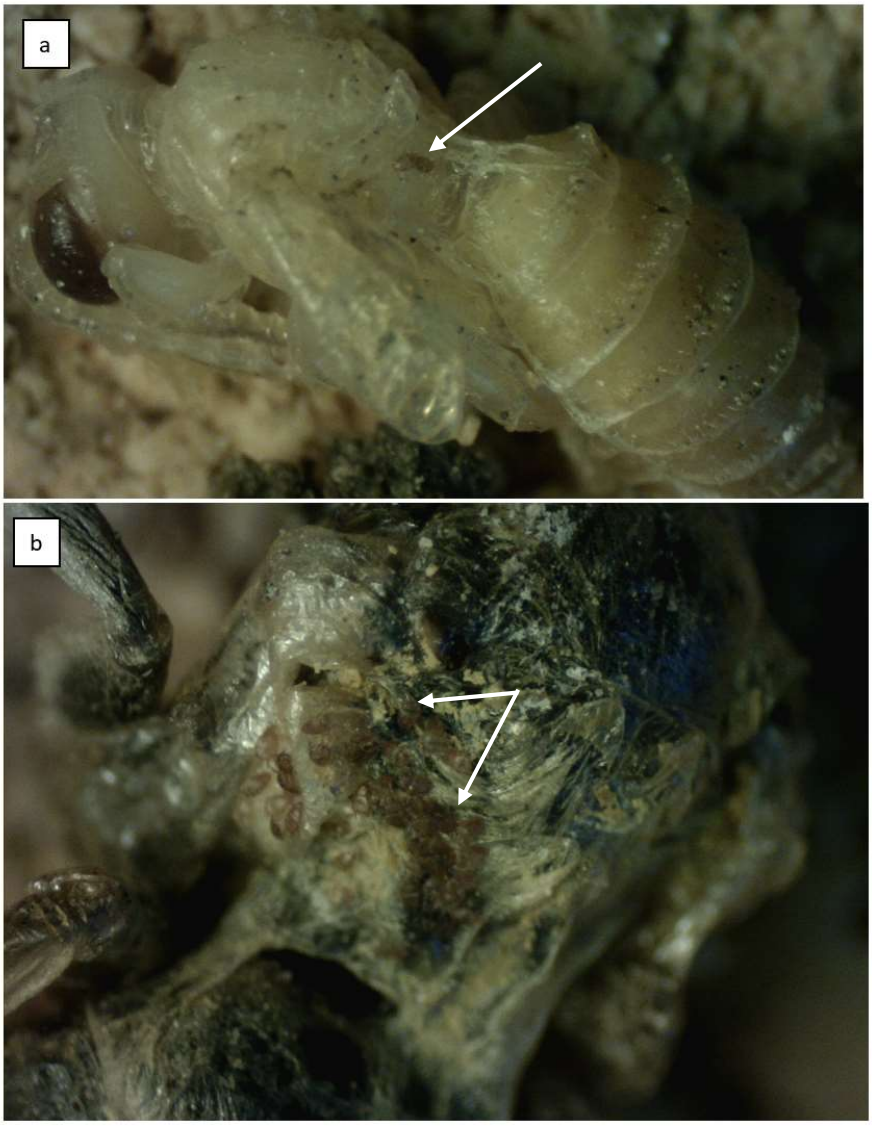


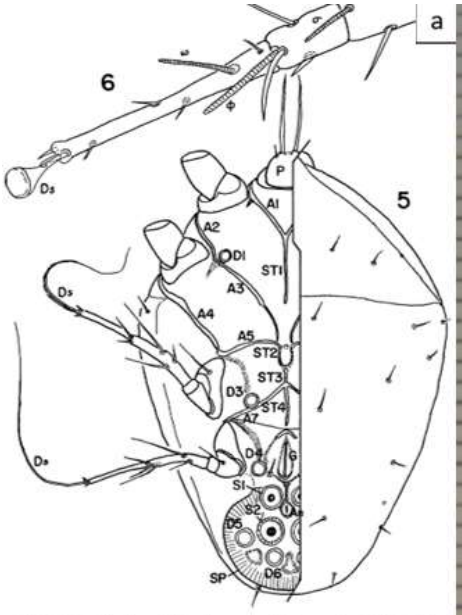
Fig 3.22. Deutonymphs of *Anoetus halictonida* on male pupae of *Halictus rubicundus*. a) Pupa with pigmented eyes and lacking further sclerotization; mites (arrow) present on propodeum. b) Heavily sclerotized pharate individual with mites present on propodeum, bases of wings (metanotum), and wings.

The majority of mites were found on either adult bees, or the post-larval immature stages. However, all individual mites believed to be the adult females of *A. halictonida* were collected from bee larvae or the brood cells of bee larvae. Only 12 bee larvae were observed with mites in direct contact, but five more bee larvae were found with large, adult females residing on the cell wall. The majority (83.3%) of the bee larvae had only one adult female mite detected, whereas the other two bee larvae (16.7%) had two mites or 11 mites, which all appeared to be adult females.

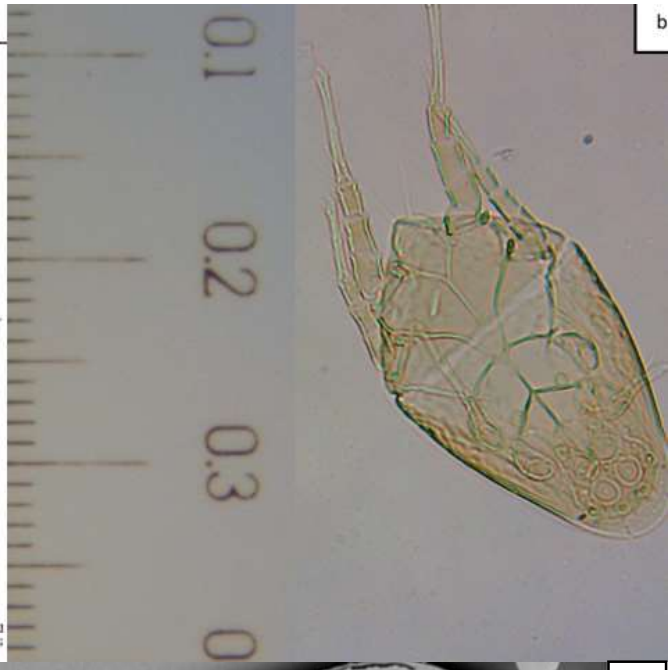
3.3.3.4 Method of attachment

Deutonymphs of *Anoetus halictonida* have some notable morphological features to assist them in maintaining their positions on a host body. These features, shared not just with other members of the genus, but also within the family Histiostomatidae, are distinguishing characteristics, with arguably the most obvious being the caudal suckerplate which extends nearly a third the length of the deutonymph's body (discounting the legs) (Fig 3.23a and b). The caudal suckerplate is aided in its function by other alterations to the deutonymph body, such as dorsal-ventral flattening of the body (Fig 3.24). Deutonymphs tended to have a particular orientation, the posterior two pairs of legs tucked in and anterior ends generally pointing in the same direction as their fellow mites (Fig. 3.25a), occasionally overlapping like scales in densely populated regions of their host (Fig. 3.25b). The relative positioning of deutonymphs was variable though, with some mites obviously positioned in direct opposition to their neighbours (Fig. 3.25a), which apparently depicts an individual in the act of repositioning, posterior legs extended as if turning, and anterior end oriented towards the base of the bee wing that the mite was removed from.

Those deutonymphs shown on the underside of the male bee head generally face towards the anterior end the bee (Fig. 3.25b). Whereas the posterior end of deutonymphs closely adheres to the host's surface, the mite's anterior region is elevated, such that high mite densities are achievable (Fig. 3.25b). Numerous mites can adhere to their host in comparatively small areas, with 4 to 6 deutonymphs managing to attach within a 260 μm^2 area of host integument (approximately 59.2-88.76 mites/mm^2).



FIGS. 5-6. *Histiostoma kalictonida* n. sp. Deutonymph. 5. Ventral-dorsal view; 6. Dorsal view of left leg I; Abbreviations: A-apodeme; An-anus; D-disc; Ds-terminal tarsal seta; G-genital slit; S-sucker; SP-sucker plate; ST-sternite.



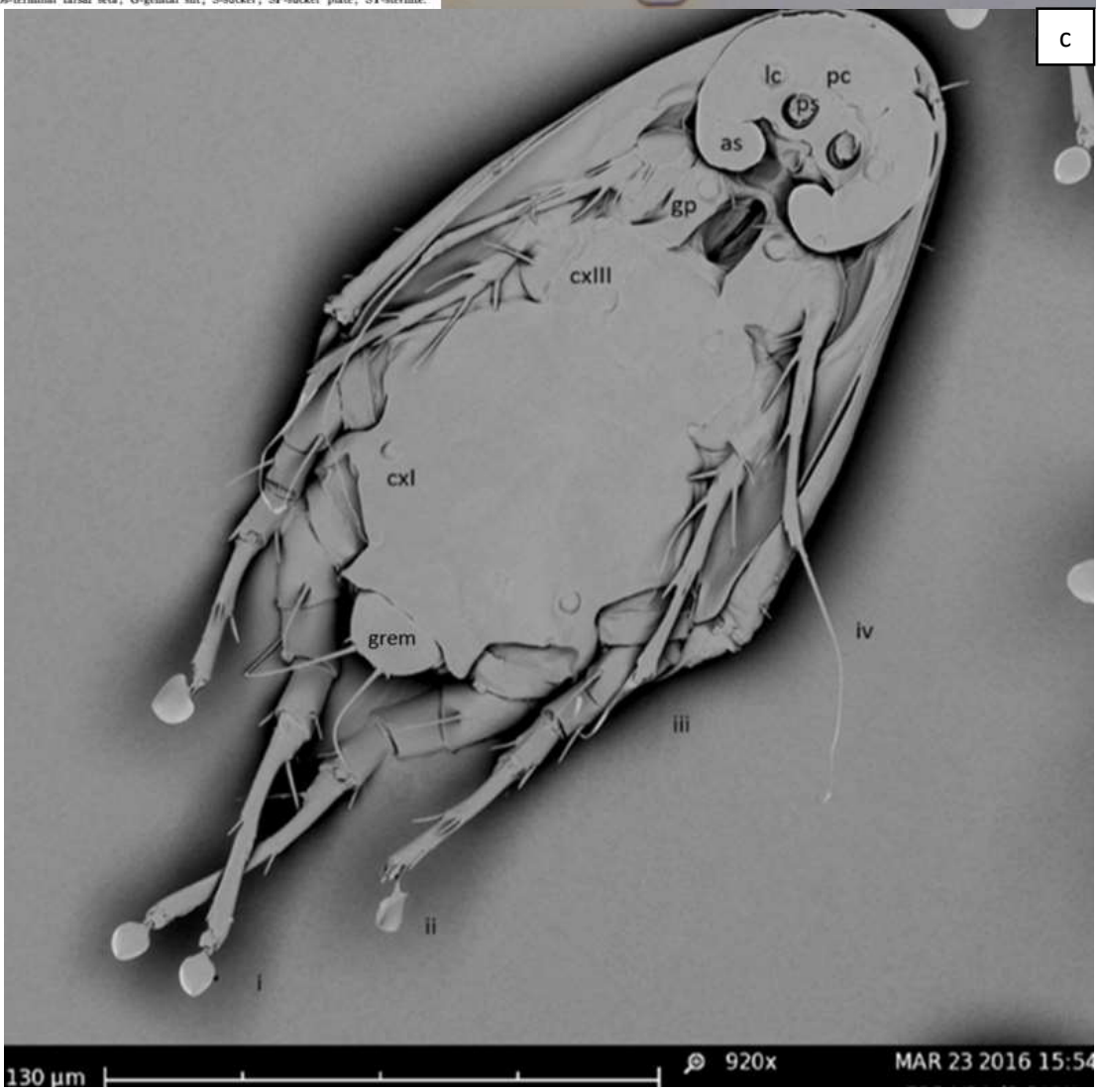


Fig 3.23. Deutonymphs of *Anoetus halictonida*. a) Line drawing by Woodring (1973) in which *Histiostoma halictonida* is first described. b) Viewed with light microscopy, scale bar with increments of 0.01 mm. c) SEM of deutonymph of *Anoetus halictonida* in ventral view illustrating terminology of Fain and Erteld (1998) for caudal suckerplate (as=anterior sucker, ps=posterior sucker, lc=lateral conoide, ps=posterior conoide), gnathosomal remnant (grem), legs (leg pair i, ii, iii, and iv) and ventral conoides (cxI, cxIII, and gp) similar in form to conoides on caudal suckerplate.

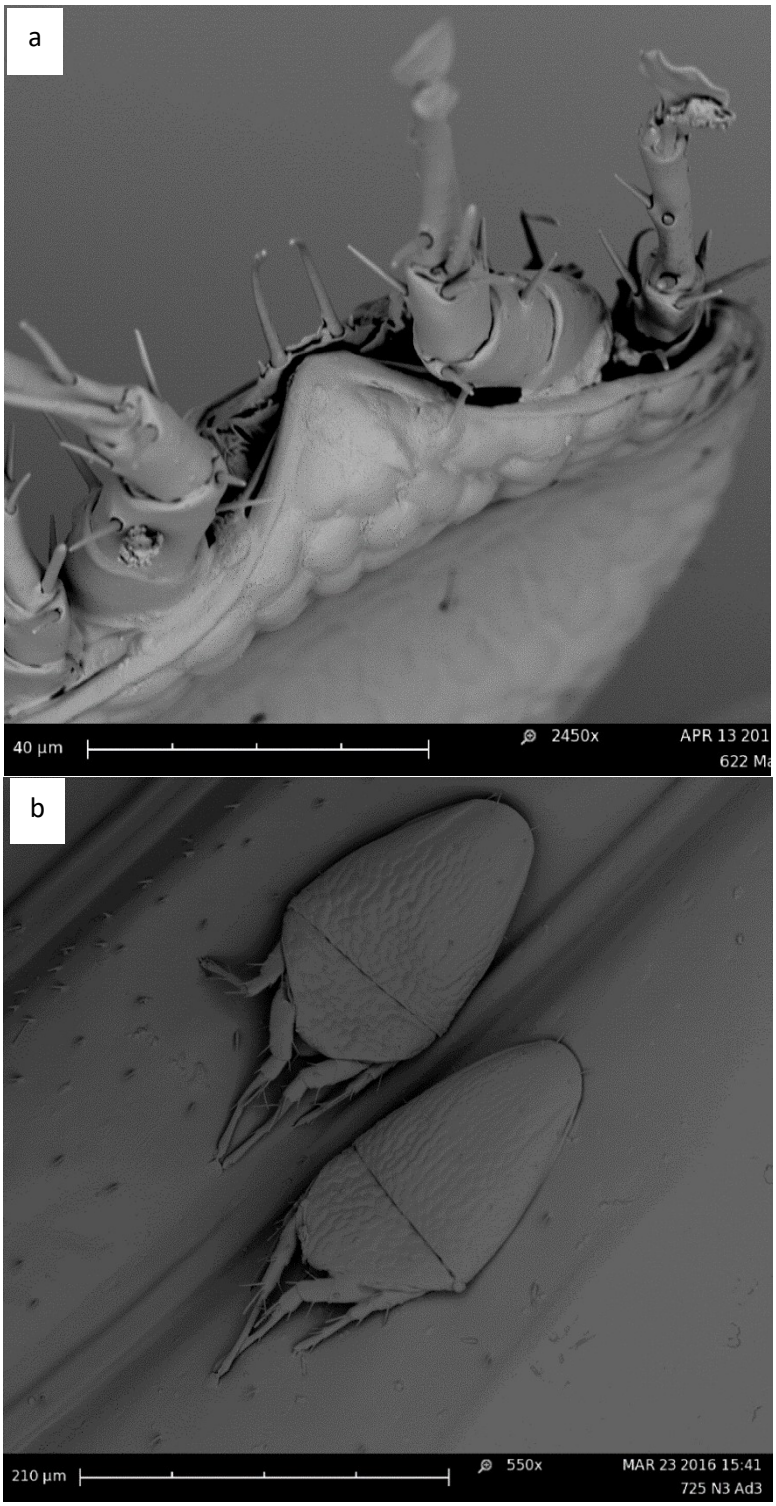


Fig 3.24. SEM showing the typically dorsal-ventrally flattened body of deutonymphs of *Anoetus halictonida*. a) Anterior view of gnaothsomal remnant and dorsal shields. b) Mites laying flat on lower surface of a fore wing.

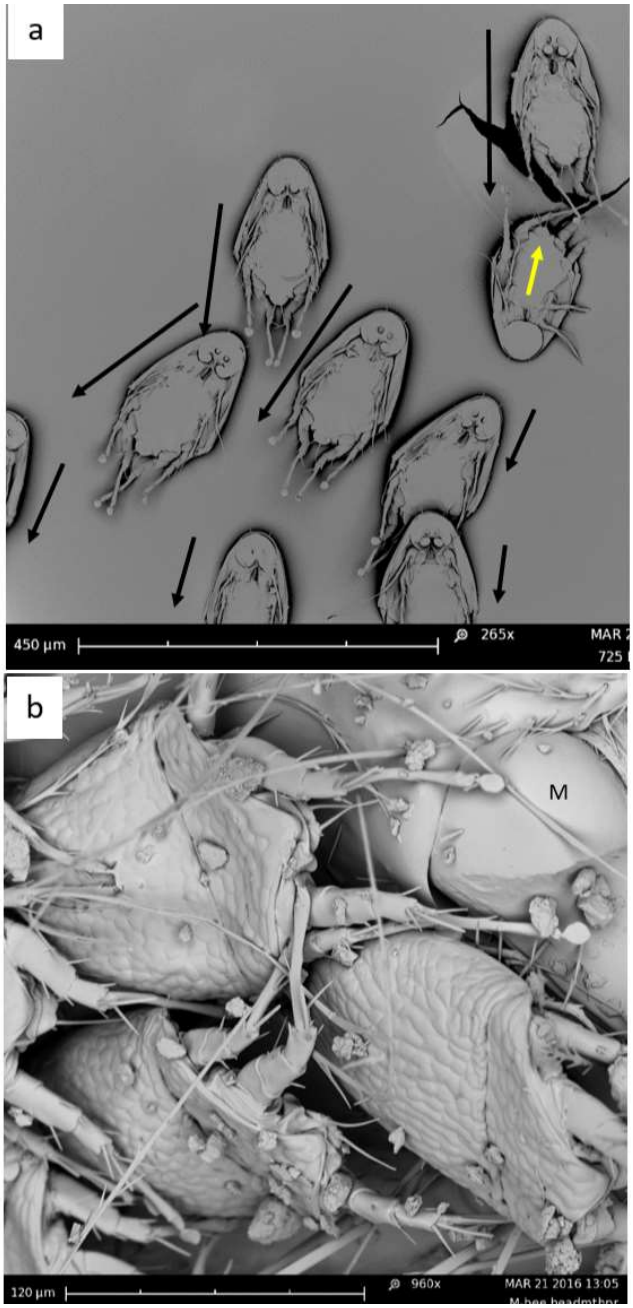


Fig. 3.25. SEM of deutonymphs of *Anoetus halictonida* collected on adult bees of *Halictus rubicundus* during nest excavation at site 1. a) Deutonymphs viewed ventrally in their original placement on the lower wing surface of a female after adhering to the SEM stub's sticky tab following gentle pressure to the wing. Arrows denote relative orientation of deutonymphs, with the majority directed towards the wing tip. b) Overlapping deutonymphs residing on the underside of the head of a male bee at the base of the right mandible (M).

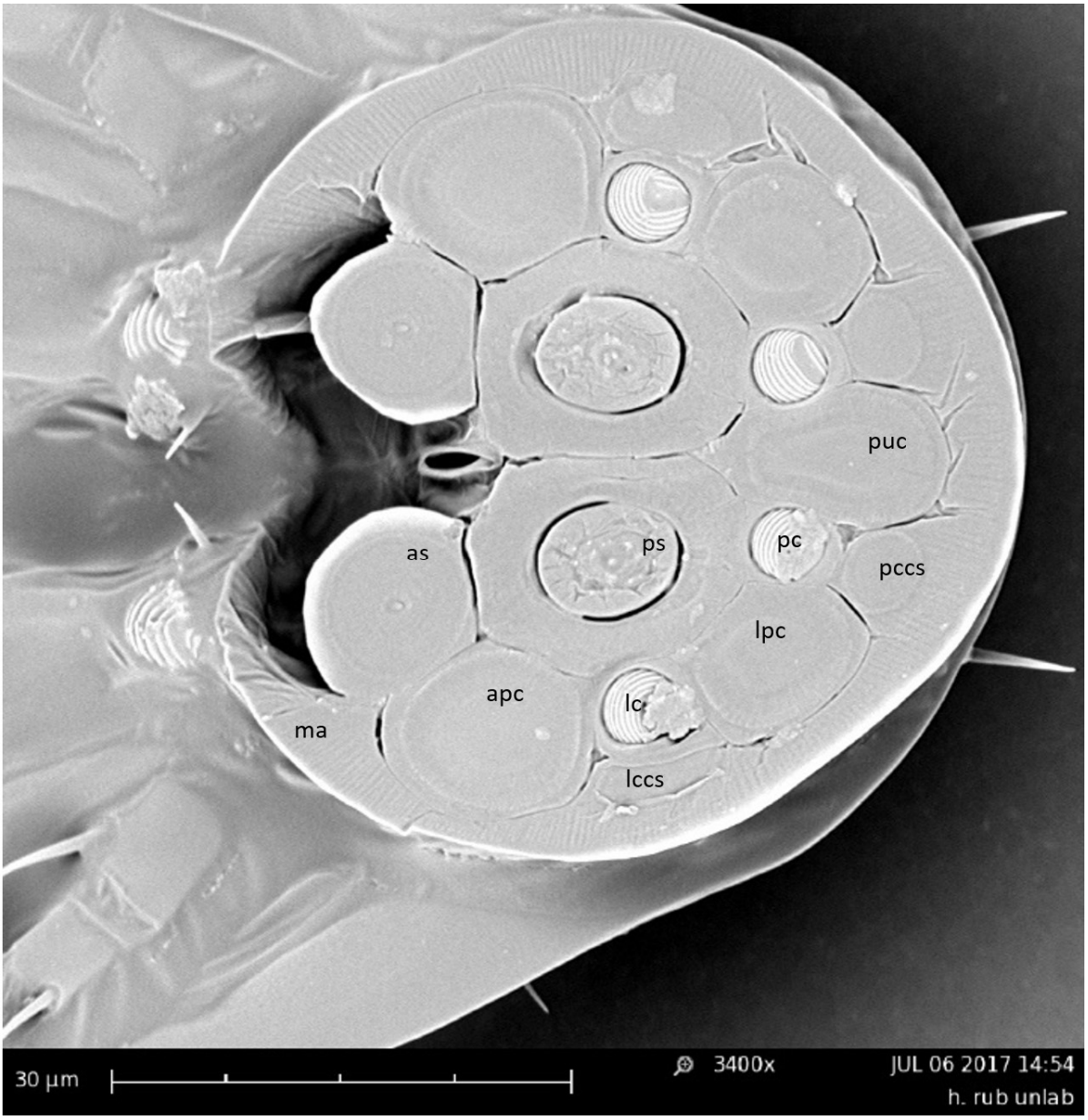


Fig 3.26. SEM of caudal suckerplate of deutonymph of *Anoetus halictonida* removed from adult female of *Halictus rubicundus*, and labelled to illustrate its component structures. Anterior suckers aligned with wing-like extensions of suckerplate, pulled away from vertical axis of the mite. Terminology is a combination of Fain and Erteld (1998):as=anterior sucker, ps=posterior sucker, pc=posterior conoide, lc=lateral conoide, and Klimov et al. (2004): apc=anterior cuticular sucker (paired), lpc=lateral cuticular sucker (paired), and puc=posterior cuticular sucker (unpaired). Also included are the abbreviations ma=mantle or edge of the suckerplate,

lccs=apparent cuticular sucker associated with lateral conoide, and pccs= apparent cuticular sucker associated with posterior conoide.

The caudal suckerplate is textured with multiple, uniformly-spaced ridges at its circumferential margins comprising the mantle (Fig 3.26, 3.30), and the plate's main components are four suckers (anterior and posterior in two pairs) and four discs/conoides (lateral and posterior, also in two pairs) (Fig 3.23c, 326), somewhat separated by smoother, flattened regions. The suckerplate is demonstrably flexible (Fig 3.27a-d, 3.28a-d, 3.29a,b). The suckerplate is variable in shape, occasionally appearing as a flattened irregular plate with the individual suckers and discs being difficult to distinguish (Fig 3.27a, 3.28a-b), at other times with the movable S1 (Woodring 1973 terminology) or anterior (*as)* suckers extended (Fig 3.29b) and wing-like edges of the anterior suckerplate withdrawn from the centre of the body (Fig 3.27b-d, Fig 3.28c-d), exposing a small pore, the anus (according to Woodring 1973; see Fig. 3.23a) located near the center of the suckerplate (Fig 3.36, 3.27d). The two posterior (*ps*) fixed suckers (Fig 3.23c, 3.26) near the middle of the suckerplate look partially deflated in certain views (Fig 3.28c, 3.29a) compared to other views in which the entire suckerplate appears flattened (Fig 3.28b, 3.30). Certain areas, specifically those parts of the caudal suckerplate between the conoides and suckers proper can be difficult to distinguish at low magnification, but are distinct (Fig 3.26). There are additional plate microsctructures (lccs, pccs) adjacent to the conoides that appear between the conoides and the striated, flexible mantle (Fig 3.26). These areas are distinct and similar to and tentatively referred to as cuticular suckers that are more apparent, but do not appear in Woodring's description of the morphology of the caudal suckerplate of *A. halictonida* (Fig 3.23a), likely a limitation of the microscopic instruments available at the time. Although the suckers have a fairly obvious role in attachment, there is some consideration as to the purpose of the conoides. It has been hypothesized that the conoides are releasing organs, facilitating the detachment of the caudal suckerplate (Fain and Erteld 1998). The conoides were observed in both collapsed and erected states, leading to the idea that conoides act as elastic buffers. The authors also make note that within the genus *Histiostoma* species have five pairs of conoides, whereas some other genera have conoides only on the suckerplate. At the time of publishing, the article refers to *Histiostoma halictonida* and several other mites that may be, or have already been, reclassified into *Anoetus*.

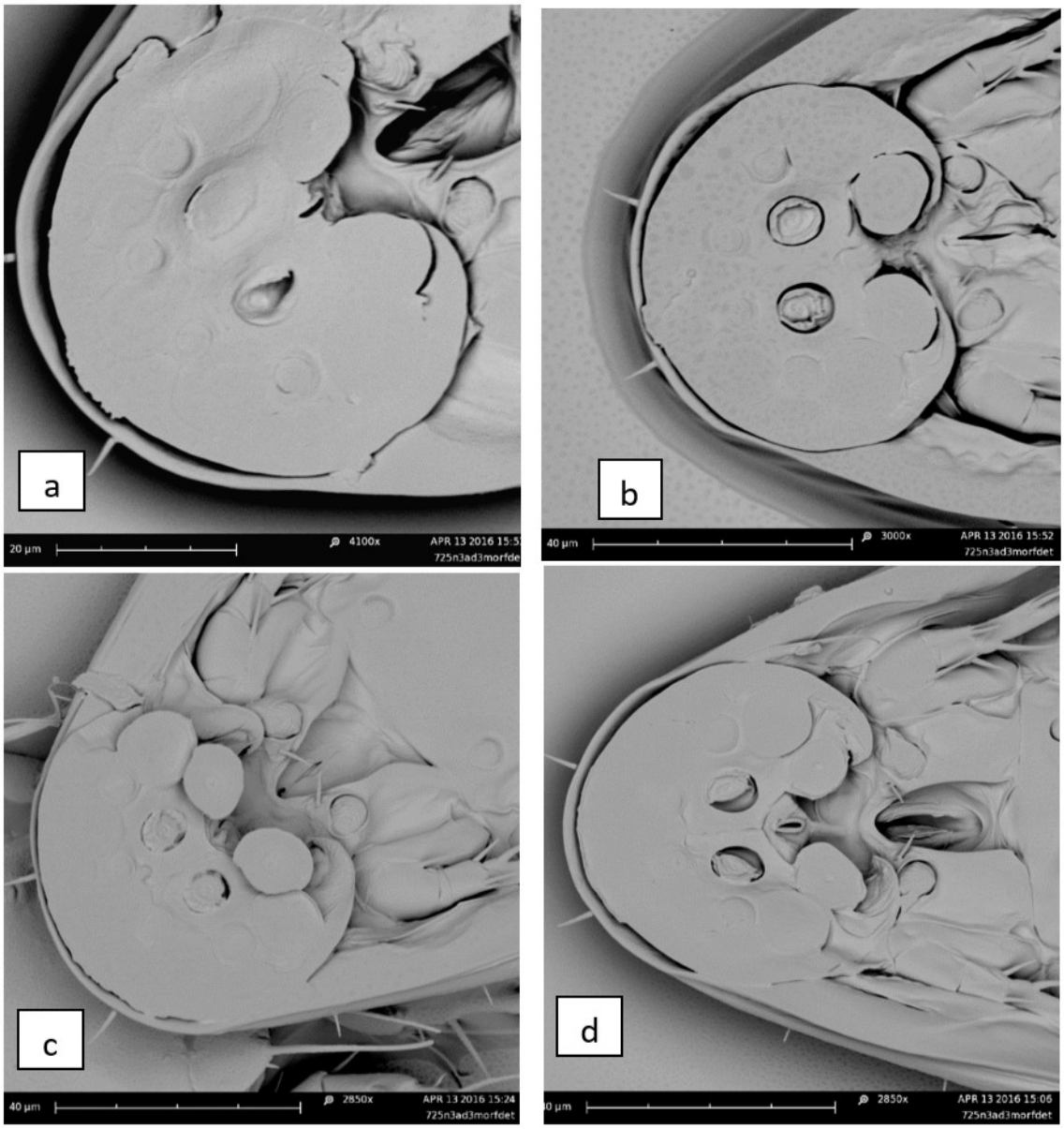


Fig 3.27. SEM of caudal suckerplates of deutonymphs of *Anoetus halictonida* removed from the lower wing surface of an adult female of *Halictus rubicundus* (collected July 25, 2015) during nest excavations at site 1. Slight variations in shape of attachment organ and position of its suckers are evident.

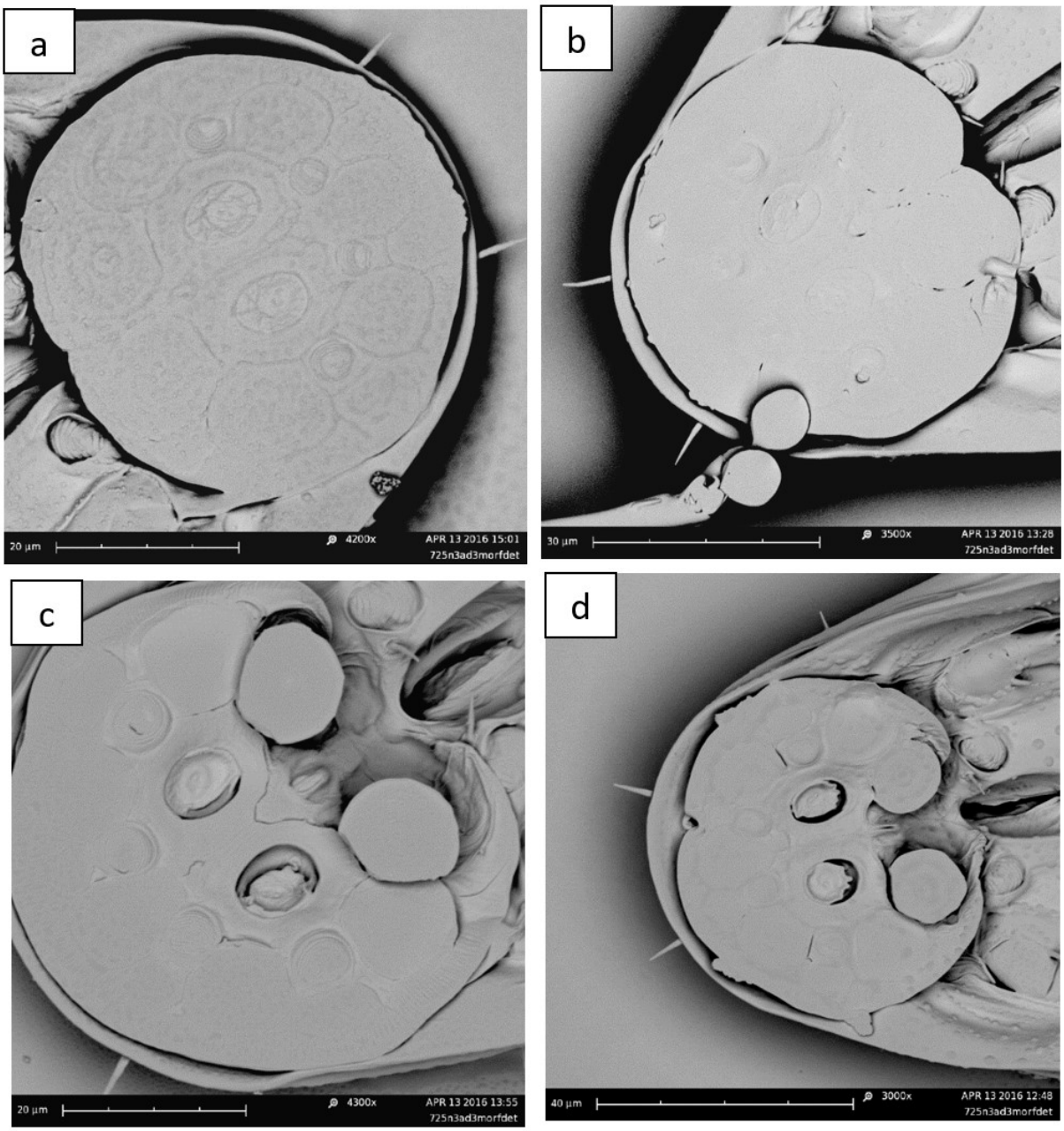


Fig 3.28. SEM of caudal suckerplates of additional deutonymphs of *Anoetus halictonida* removed from the lower wing surface of an adult female of *Halictus rubicundus* (collected July 25, 2015) during nest excavations at site 1. Note variations in shape of attachment organ, conoides, and suckers.

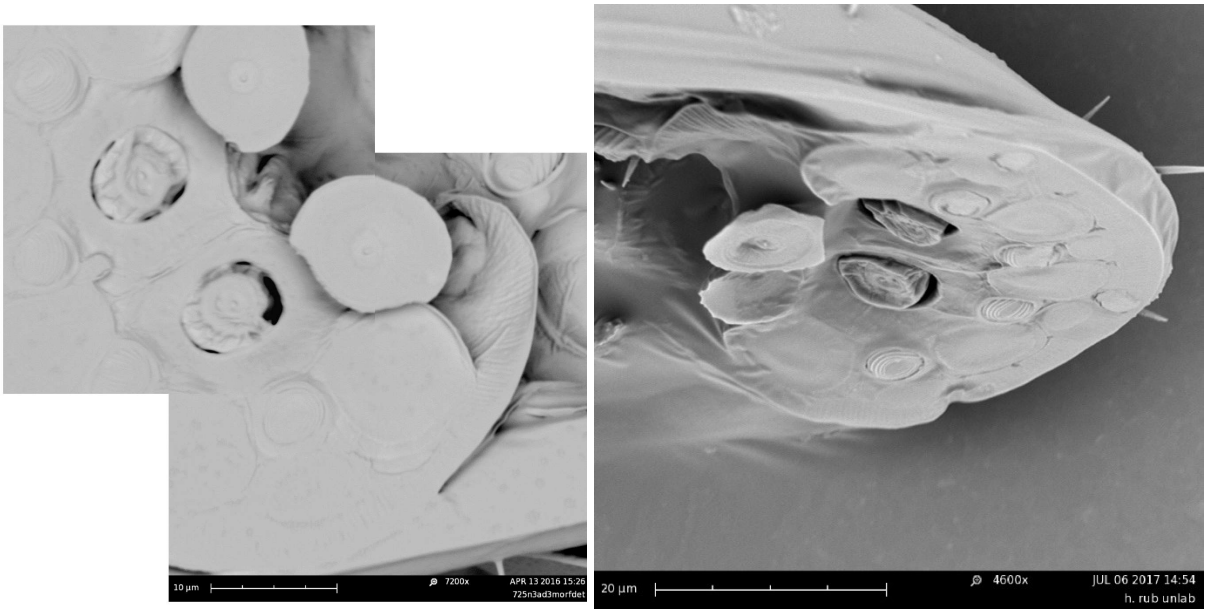


Fig 3.29. SEM of caudal suckerplates of deutonymphs of *Anoetus halictonida* removed from separate adult females of *Halictus rubicundus* during nest excavations at site 1 showing differing views of anterior and posterior suckers. a) Composite of SEM images of caudal suckerplate of deutonymph of *Anoetus halictonida* showing anterior suckers raised and separated from wings of plate, and posterior suckers partially collapsed. b) Oblique view of caudal suckerplate showing extended anterior and posterior suckers.

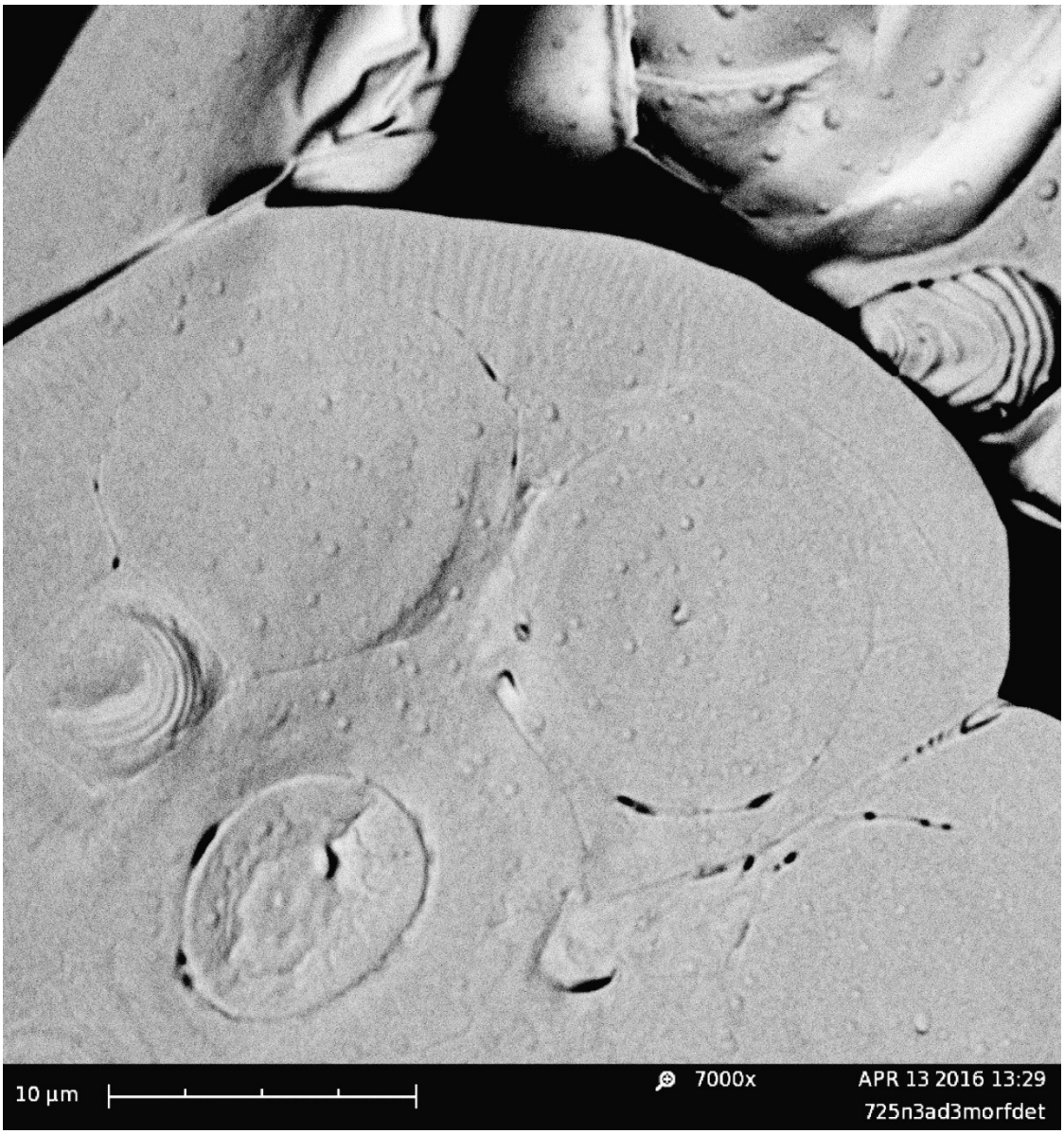


Fig 3.30. SEM of deutonymph of *Anoetus halictonida* showing flattened caudal suckerplate with expanded posterior suckers and anterior suckers aligned with the wing-like extensions of the suckerplate, which have been folded in to meet in the centre. Note ridged nature of the right lateral conoide (at left), and faint ridges along circumference of the plate (at top). Small scattered bumps are technical artifacts.

Whereas the caudal suckerplate is the obvious structure involved in attachment of the deutonymph to its host, the leg segments also appear to contribute to positioning on the bee body. It should be noted there are slight morphological differences between leg pair i and leg pair ii, such as the shape of the empodia at the tips (Fig 3.31a), appearing circular in leg pair i (Fig 3.31a,d) and broader on leg pair ii (Fig 3.31a,c). The first two pairs of legs are obvious at all times, often slightly reflexed and extended in front of the mite (Fig 3.32a). When deutonymphs reside on a bee, these structures can be seen placed flat and tense against the bee body (Fig 3.32a), with slight striations visible at high magnification, appearing almost like a clam shell (Fig 3.32b).

3.3.4 Mite stages and their morphology

In addition to the especially common deutonymphs, gravid adult females (Fig 3.33), and tritonymphs (Fig 3.34, 3.35b,c) were recovered during the nest excavations (2015). Other mites, possibly immature, non-gravid females or tritonymphs, were located during the excavations (Fig 3.36), but a lack of literature on their appearance means that their specific stage (and even whether they are *Anoetus halictonida*, despite the absence of other mites) is uncertain. It should be noted that the mouthparts are some of the most distinctive features of these stages (Fig. 3.35a-c), and appear almost shovel or scoop-like when viewed dorsally (Fig 3.37a,b, 3.38a-d).

3.3.5 Mite weights and phoretic loads

Table 3.3 Pooled and average weights of deutonymphs of *Anoetus halictonida* transferred manually from host bees of *Halictus rubicundus*.

Number of mites pooled before reading stabilized on microscale	Weight of pooled sample (µg)	Average mass of a deutonymph (µg)
7	4	0.571
22	9	0.409
6	4	0.667
14	5	0.357
13	10	0.769

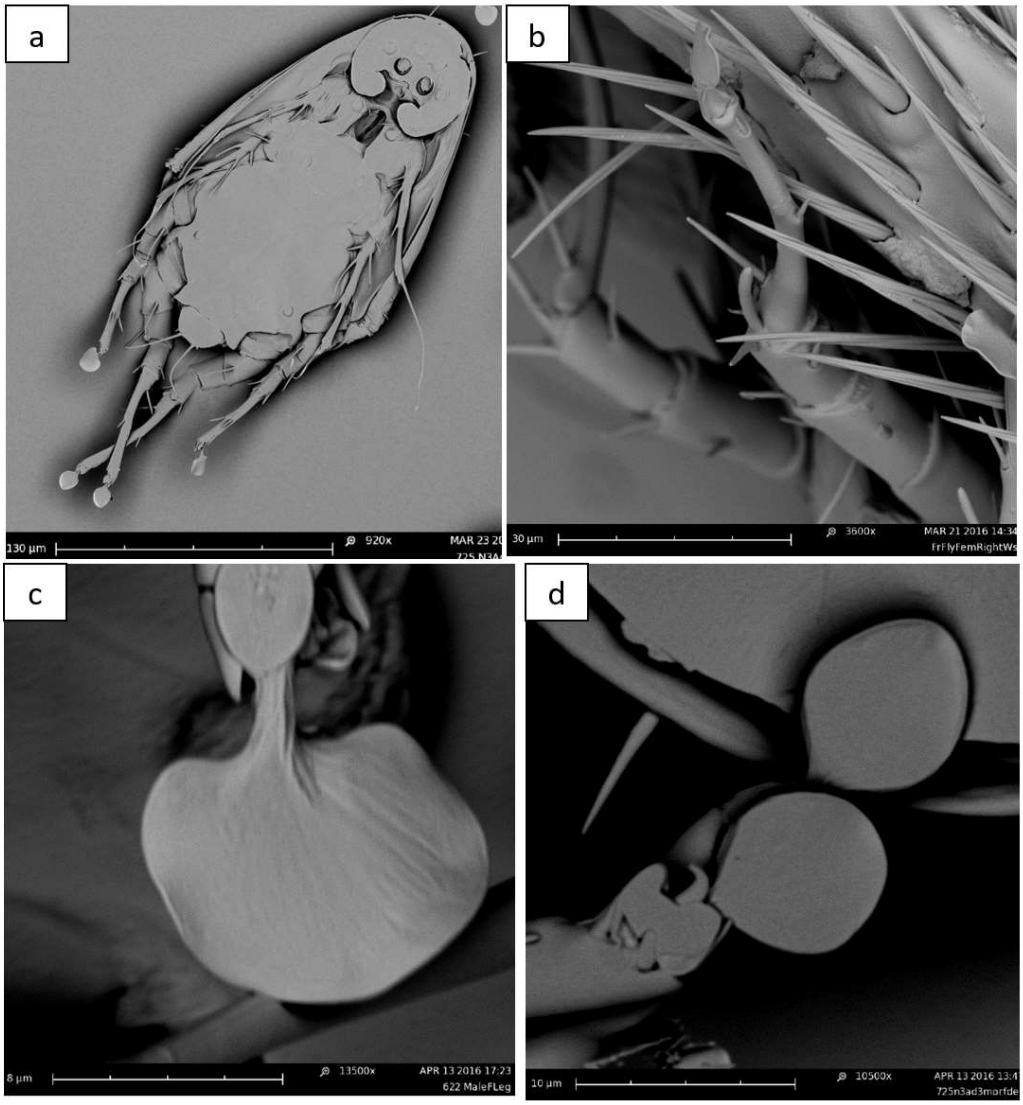


Fig 3.31. SEM of fore leg segments and empodia of *Anoetus halictonida* deutonymphs collected on *Halictus rubicundus* during nest excavations at site 1 during spring-summer 2015. a) Ventral view of deutonymph showcasing morphological differences between fore and hind legs. b) Fore legs of deutonymph present on wing of a female bee. c) Empodium of leg ii. d) Empodia of leg pair i.

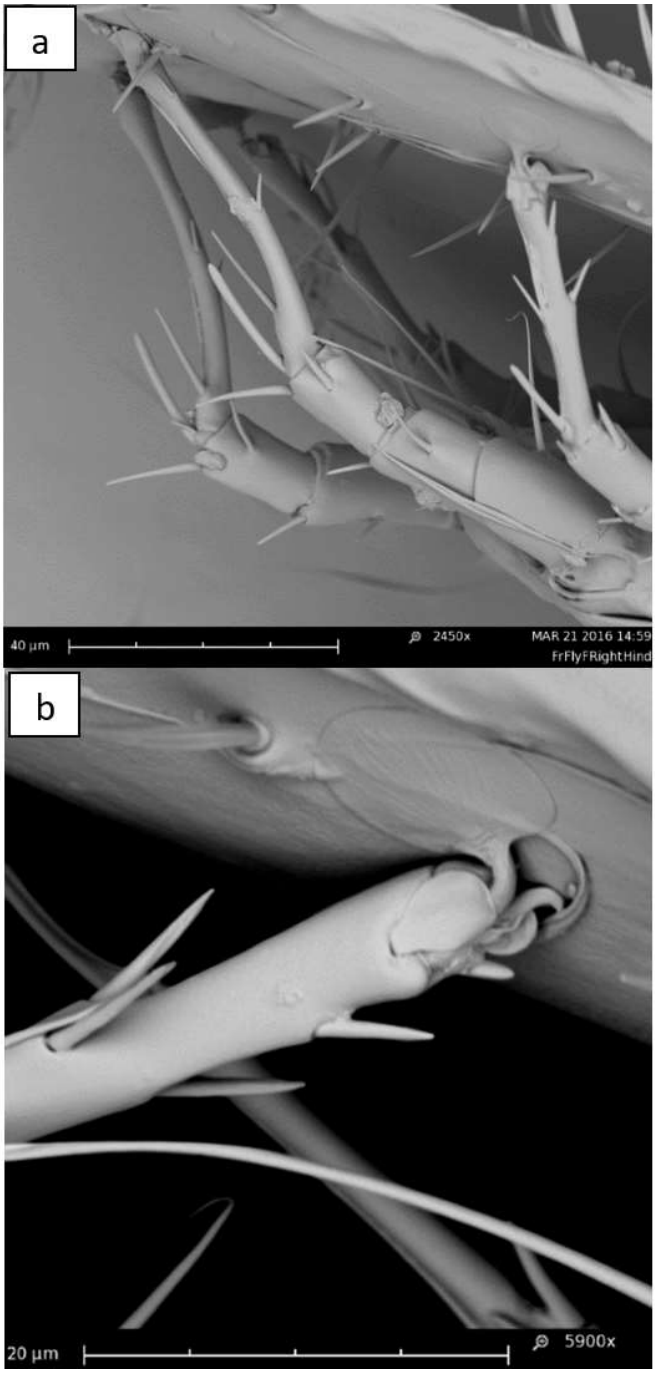
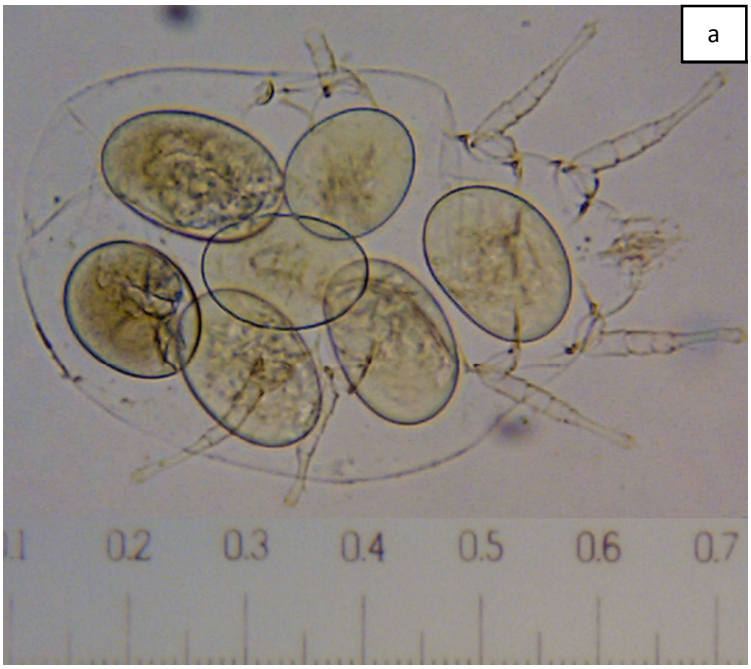
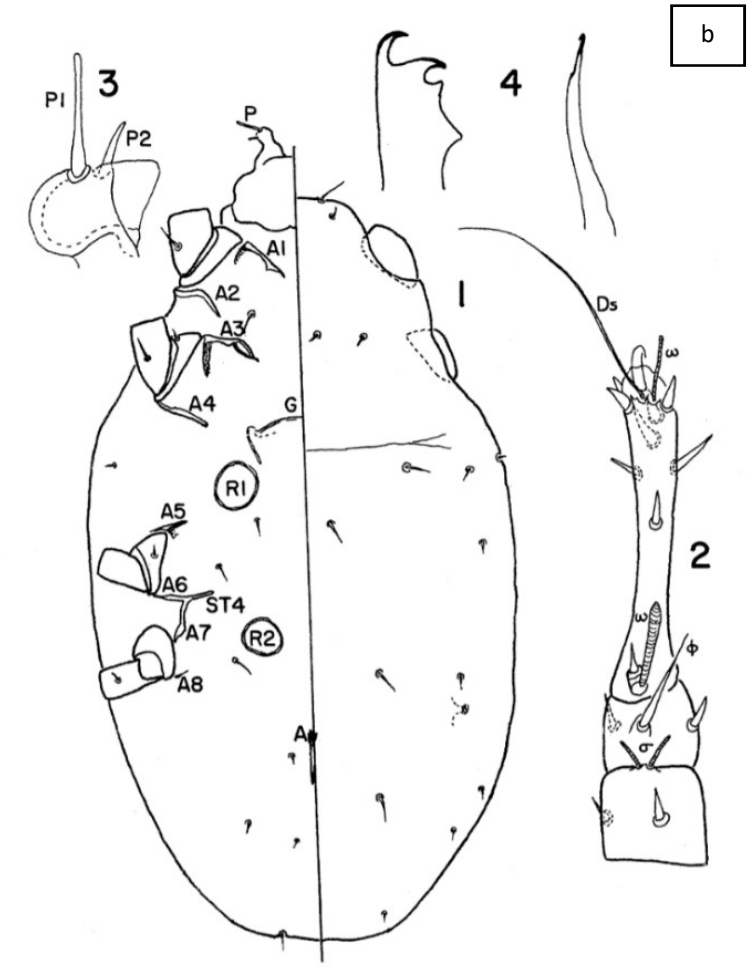


Fig 3.32. SEM of deutonymphs of *Anoetus halictonida* present on the wings of *Halictus rubicundus* collected during nest excavations at site 1 during 2015, showing the position of the leg segments when attached. a) Anterior legs with segments slightly bent and leg tips adhering to wing. b) Flattened empodium of leg of leg pair ii shown against wing surface with outline of seta visible underneath.





FIGS. 1–4. *Histiostoma haliconida* n. sp. Female 1. Ventral-dorsal view; 2. Dorsal view of left leg I; 3. Right pedipalp; 4. Lateral (left) and dorsal (right) view of distal cheliceral digit; Abbreviations: A-apodeme; An-anus; Ds-terminal tarsal seta; G-genital slit; P-pedipalp; P1 & P2-pedipalpal setae; R-chitinous ring; ST-sternite.

Fig. 3.33. Adult female of *Anoetus halictonida*. a) Gravid female collected on a larva of *Halictus rubicundus* located in a nest excavated on May 30, 2015. This adult female is relatively large, approximately 0.55 mm in length, not including the legs (scale bar increments of 0.01 mm) and colourless or white except for its eggs or oocytes (the 7 dull yellow spheres within the abdomen). Note the mite's well developed mouthparts and hind legs, and lack of a caudal suckerplate. b) Line drawing from original description of the species by Woodring (1973).

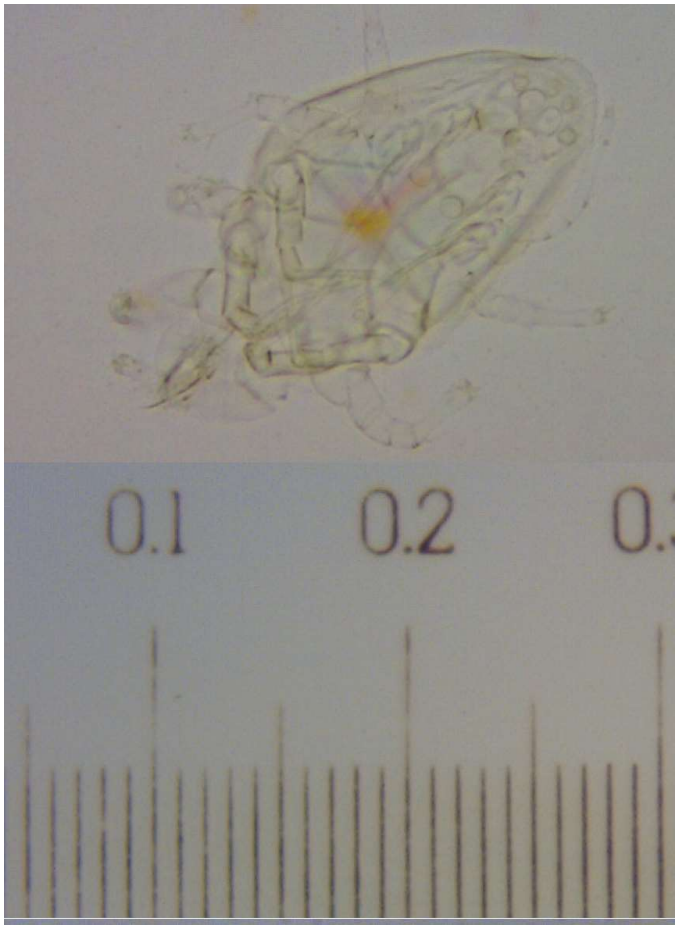


Fig 3.34. Moulting pharate *Anoetus halictonida* tritonymph emerging from (and appearing to overlap) its deutonymph stage. The tritonymph has comparatively well developed mouthparts and hind legs. Scale bar with increments of 0.01 mm.

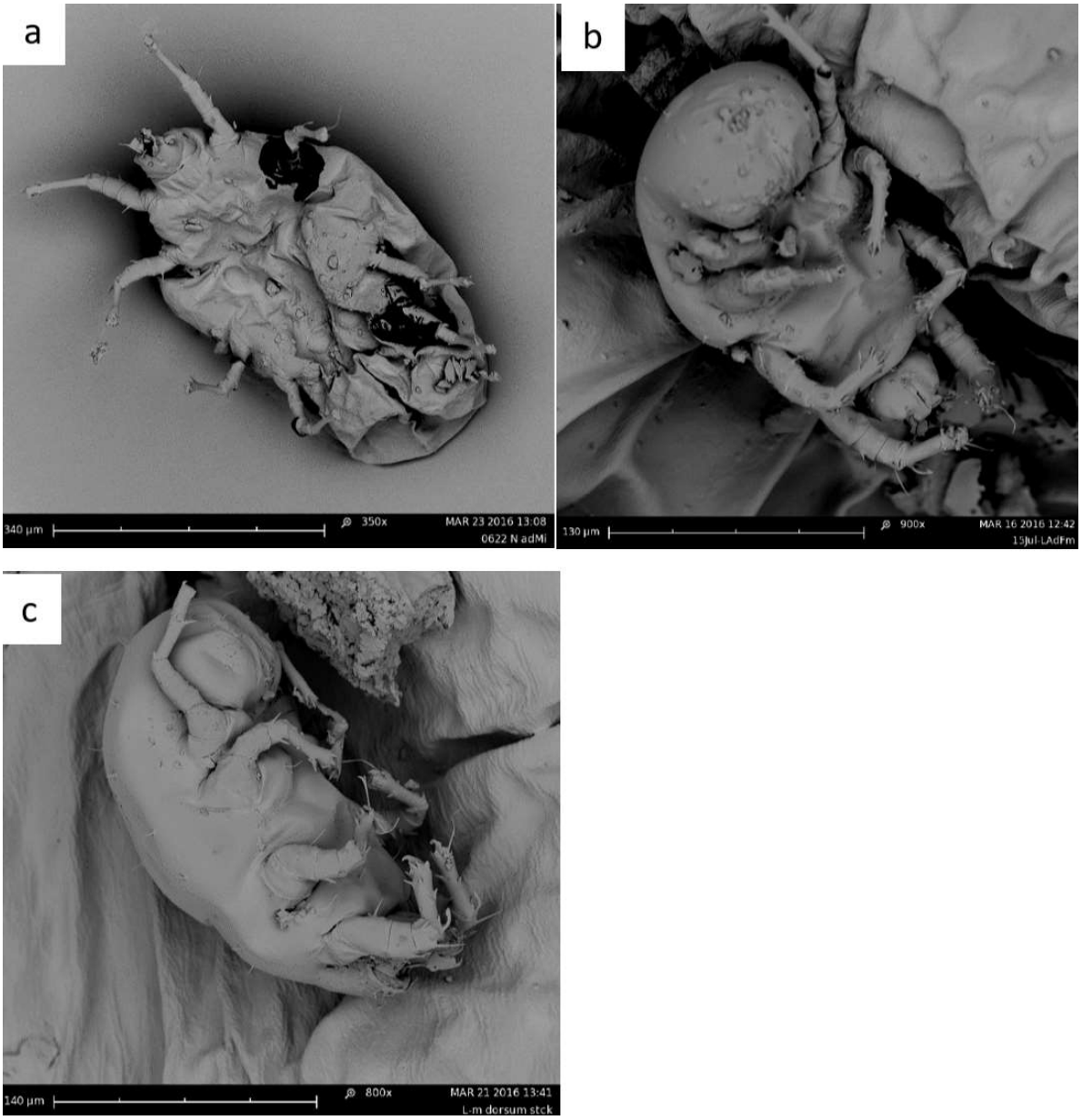


Fig 3.35. Apparent post-deutonymph stages of *Anoetus halictonida* collected during nest excavations during 2015. a) Ventral view of an adult female, noticeably larger than the other mite stages. b) Ventral view of an apparent tritonymph on the integument of a larva of *Halictus rubicundus*. C) Lateral view of an apparent tritonymph on integument of a larva of *H. rubicundus*.

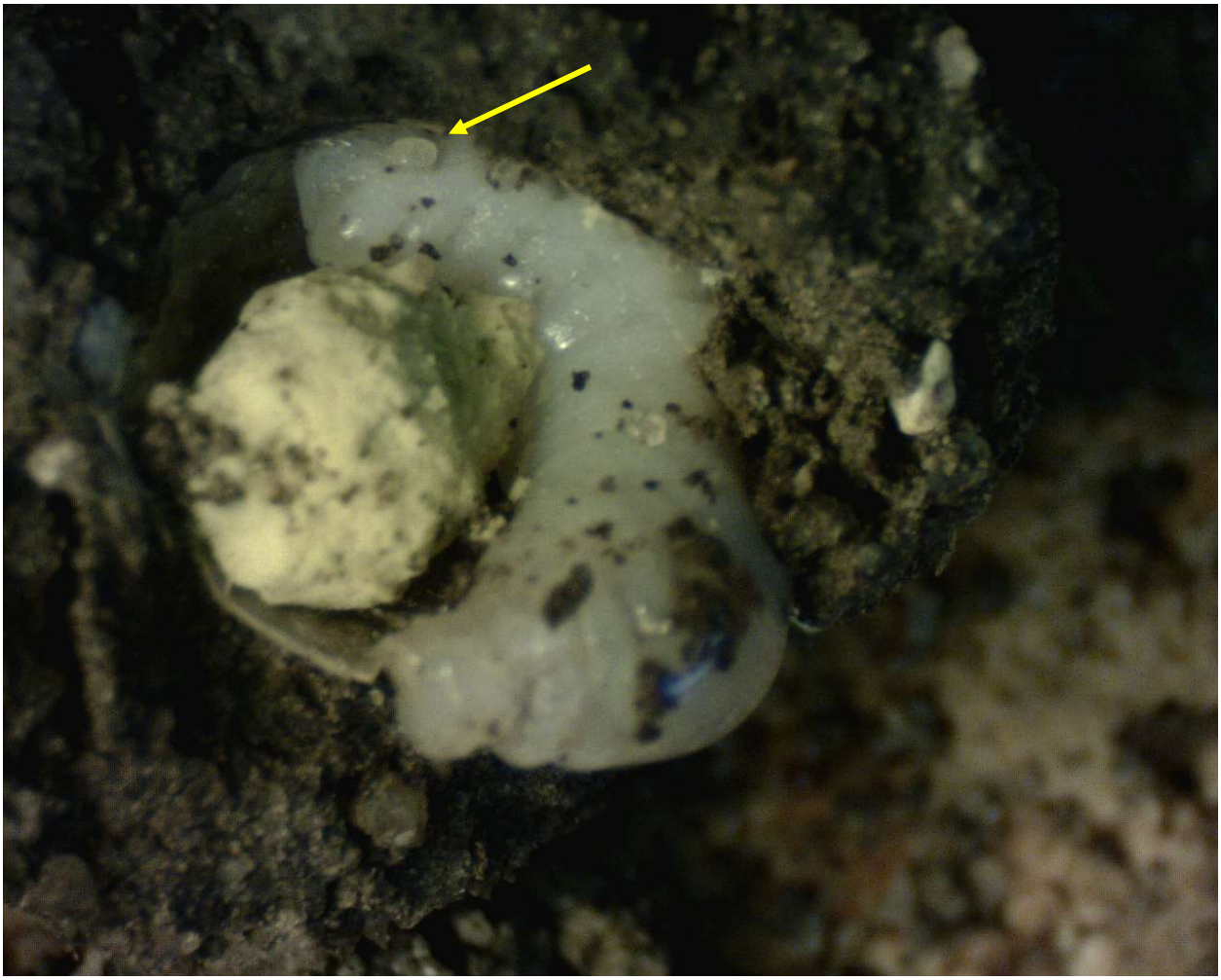


Fig 3.36. *Halictus rubicundus* larva curled around its pollen provision, depicting a large adult female mite of *Anoetus halictonida* present on its posterior. This immature bee was collected during a nest excavation at site 1 on June 1, 2015.

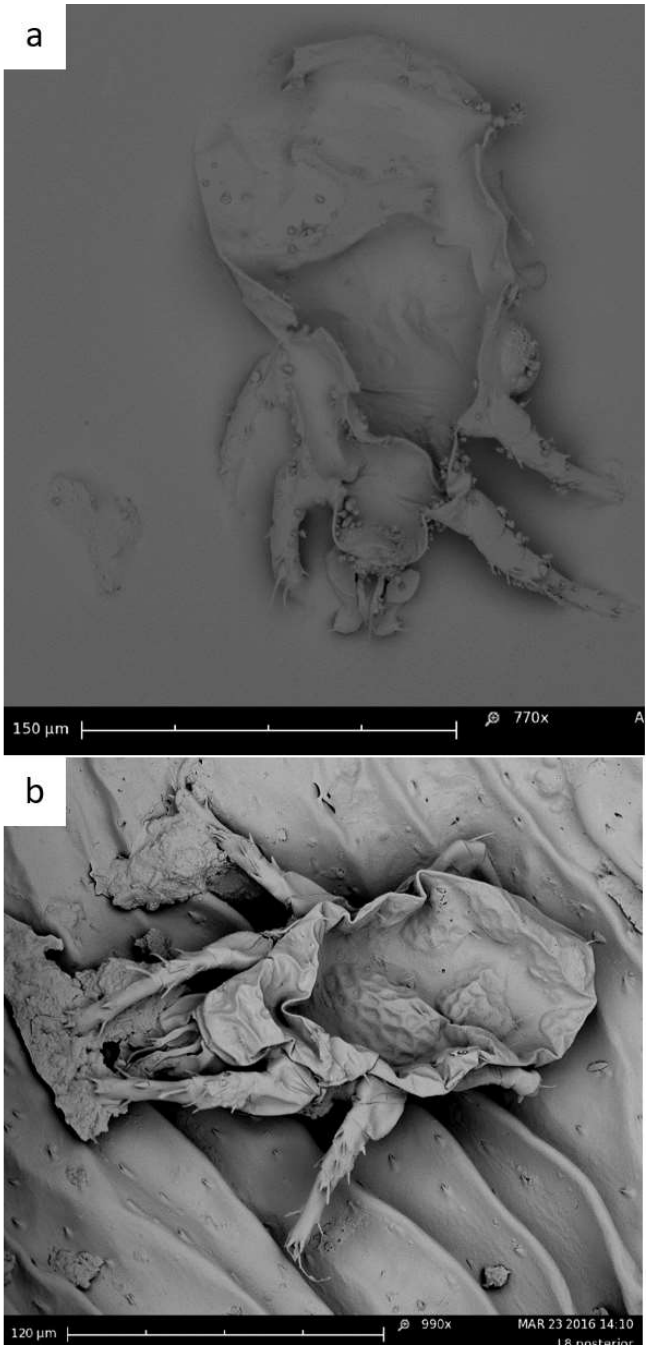


Fig 3.37. SEM images of possible tritonymphs of *Anoetus halictonida* collected during nest excavations at site 1, May 2015. a) Dorsal view of mite removed from host. b) Desiccated tritonymph (or possibly non-gravid female) on the removed integument of a larva of *Halictus rubicundus*. Dorsal view, possibly caught in the act of feeding.

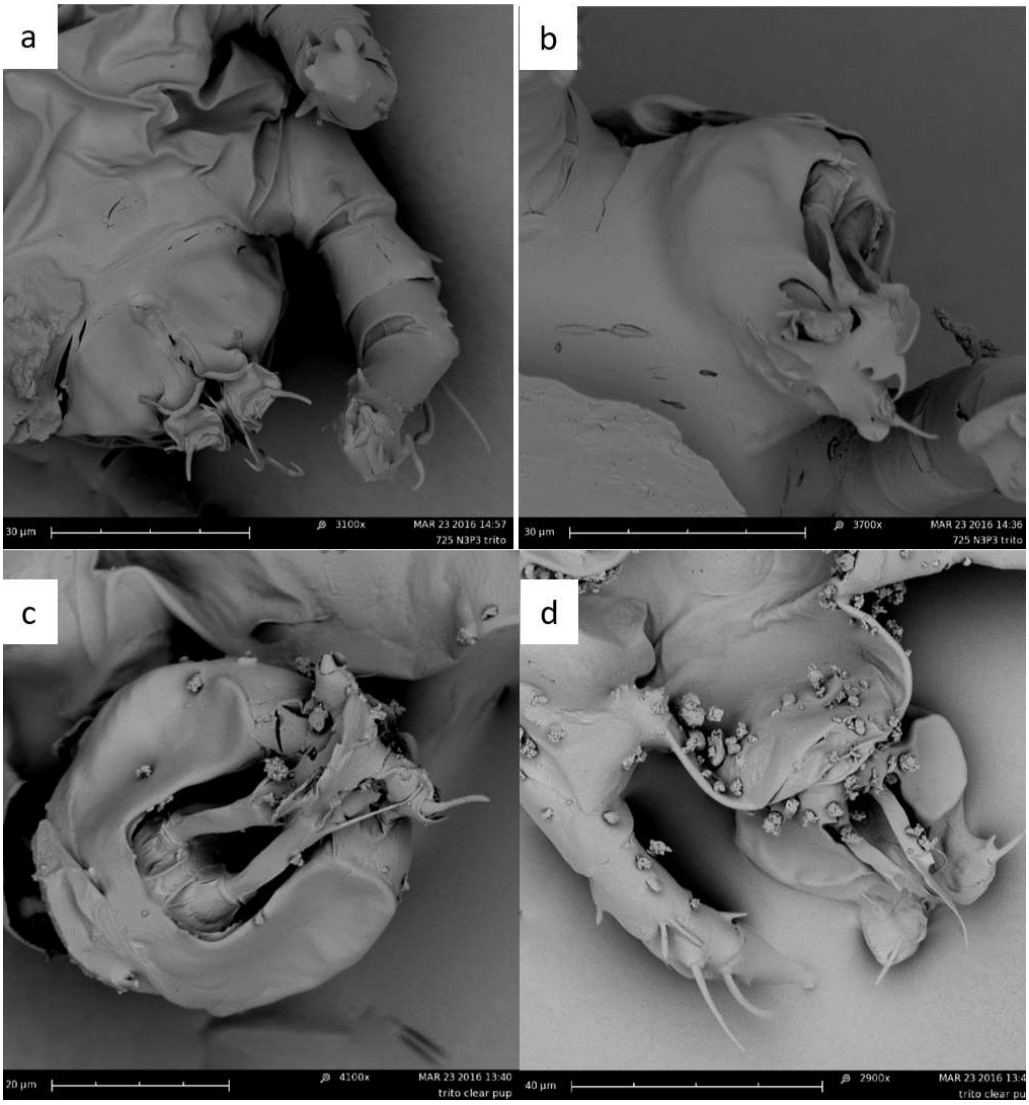


Fig 3.38. SEM photos of the mouthparts of post-deutonymph *Anoetus halictonida* mites. a) Ventral view. b) Ventral view. c) Anterior view of a twisted head (artifact of specimen mounting). d) Dorsal view.

From Table 3.3, the average mass of a single deutonymph of *A. halictonida* was 0.56 µg. The average number of mites residing on adult female *Halictus rubicundus* was 53.1, yielding an average mite load of 29.4 µg. Because the average weight of a female bee (n=4) was 13.094 mg, the mean phoretic load of deutonymphs of *A. halictonida* was 0.22% of an adult female bee's total weight. The average number of mites on adult male *Halictus rubicundus* was 17.2, yielding an average mass of this mite load of 0.0095 mg (9.5 µg). Therefore, accounting for the average weight (8.530 mg) of a male bee (n=4), the average load of *A. halictonida* mites represented just 0.11% of an adult male bee's total weight.

The deutonymphs of *A. halictonida* were measured from SEM photos to determine length, not including the legs, and width of the mite body. All of the deutonymphs (n=27) were found on the wings of adult female *H. rubicundus*. The average length of deutonymphs of *A. halictonida* was 190.3 µm (s.d. 12.7) and ranged from 167.3-207.4 µm. The average width of the deutonymphs was 119.5 µm (s.d. 8.8) and ranged from 106.0-143.5 µm.

3.3.6 Transfer of mites

3.3.6.1 Incidence of mite transfer to flowers

The total number of bees collected during the field seasons prior to 2015 was 391. This total includes all foraging bees caught during 2013 (Appendix 2), and bees recovered during 2014 excavations (Appendix 4) and during the pre-season phase of 2014 (Appendix 3), although only a fraction of these bees were *Halictus rubicundus* (n=50, 14%). Of the 21 *Halictus rubicundus* collected during the 2013 field season, there was one instance of a deutonymph (appearing to be a histiostomatid) being found on a collected and dissected inflorescence of dandelion (*Taraxacum officinale*). None of the other flowers (n=32) dissected (either visited by halictids or any bee) showed any evidence that flowers can act as areas for mites to transfer.

3.3.6.2 Carbon dioxide anaesthetization

None of the attempts (pairs of adult male and female bees of *H. rubicundus*; one pair of adult male bees of *H. rubicundus*; confined females of *H. rubicundus* and *Sphecodes*) demonstrated mite transfer between individuals, possibly due to low mite density on available specimens, or abnormal behaviour induced by anaesthetization and containment. On one occasion, a bee with its host mites was anaesthetized for a period of 15 min to determine roughly

how the process affected movement. The mites appeared unharmed, if somewhat sluggish as compared to mites removed from a pharate bee host that was in the process of removing its exuvium. There was one instance of the deutonymphs having been observed to disembark onto the inner surface of a glass vial after their host, a male adult bee being kept in isolation, was dosed with CO_2.

3.3.6.3 Collection of deceased bees

Approximately 20 to 30 dead bees of *H. rubicundus* were collected from site 1 as they became available. Time of death was unknown. As these bees were often only partial cadavers, it could be that several of the pieces belonged to the same bee. These fragments generally had the wings and antennae missing, and the end of the abdomen, if present, appeared to be chewed or broken open in many cases. Unless the wings were intact, mites were not found, in which case some deutonymphs could be observed in the same positions as the deutonymphs on living bees. Thus, not all deutonymphs depart a deceased host of *H. rubicundus*.

3.3.7 Comparison to other mites found on local halictid bees

There were several other mites of interest found on other halictid bee species in the vicinity of site 1. Occasionally seen entering the nests of *Halictus rubicundus* were cleptoparasitic *Sphecodes* bees (possibly *S. dichrous*), all believed to have been females of the same species. Three of the 5 adult *Sphecodes* collected (60.0%) had mites (Fig 3.39a) that greatly resembled *Anoetus halictonida* (Fig 3.39b). All of the *Sphecodes* had very low numbers of anoetid mites (1-3). These mites were almost identical, but showed small differences (e.g., the apodeme ridges; Fig 3.39a) under SEM that are not apparent when viewed using light microscopy of slide-mounted specimens, specifically more distinct ridges ventrally and with a collar (Fig 3.39a). Whether these are different species, or if the differences represent intraspecific variation, is unknown, but Drs. Klimov and OConnor (personal correspondence) have indicated they believe these mites are likely the same species. Also found on the same *Sphecodes* was a mite species of the family Scutacaridae (n=2) that is easily distinguished from the histiostomatids (Fig 3.41a,b). One scutacarid was found ventrally, just behind the hind coxae, and the other was found on the propodeum, whereas all the anoetids were found on the wings.

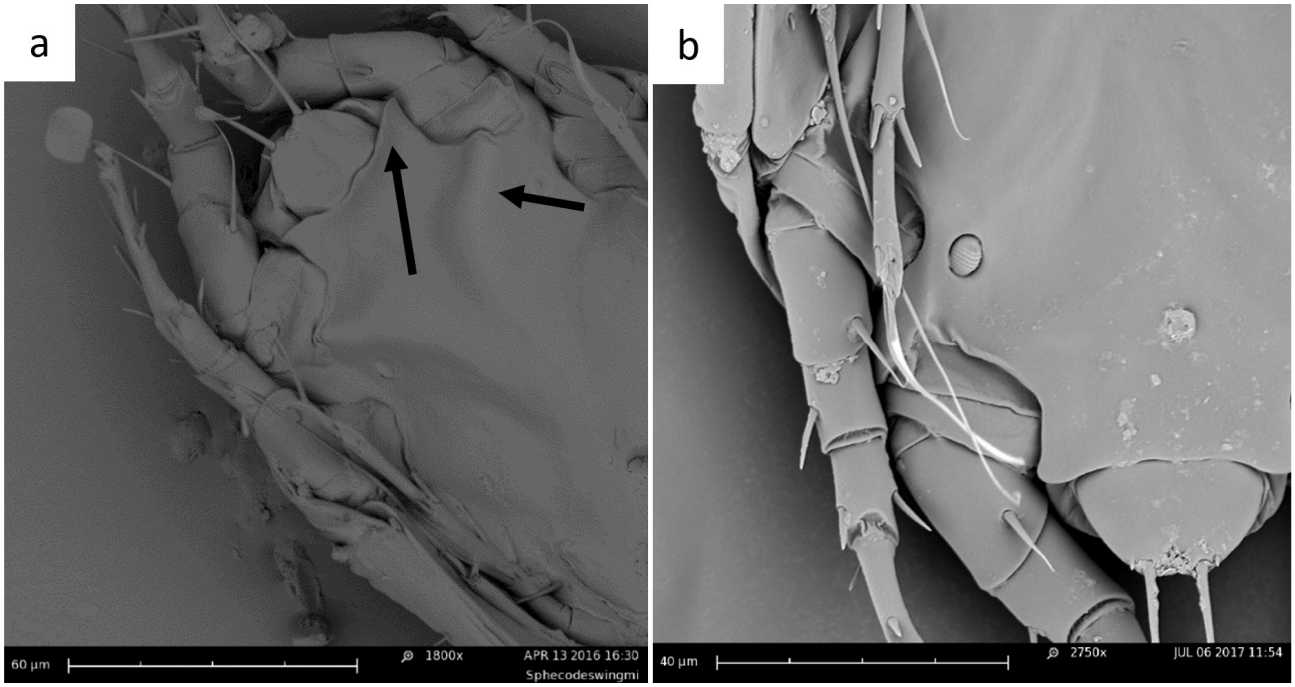


Fig 3.39. SEM comparison of mites on *Sphecodes* and *Halictus rubicundus* collected from site 1. a) Mite collected from *Sphecodes* with pronounced ridges ventrally (short arrow) and a high collar (long arrow). b) *Anoetus halictonida* mite collected from *H. rubicundus* at site 1.

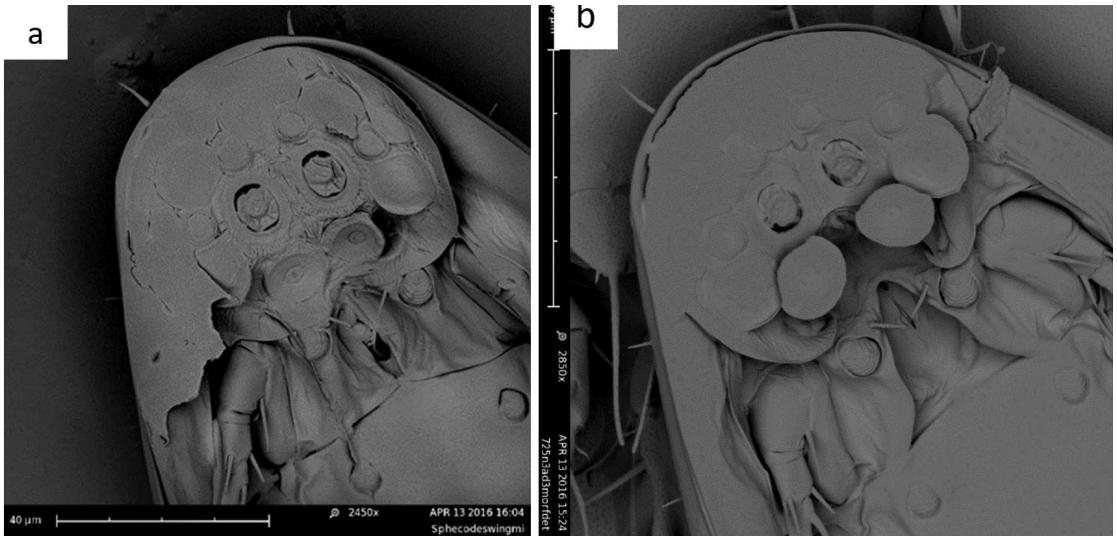


Fig 3.40. SEM image comparison of caudal suckerplates of mites on *Sphecodes* and *Halictus rubicundus* collected from site 1 in 2015. a) Caudal suckerplate of mite from *Sphecodes*. b) Caudal suckerplate of *Anoetus halictonida* from *H. rubicundus.*

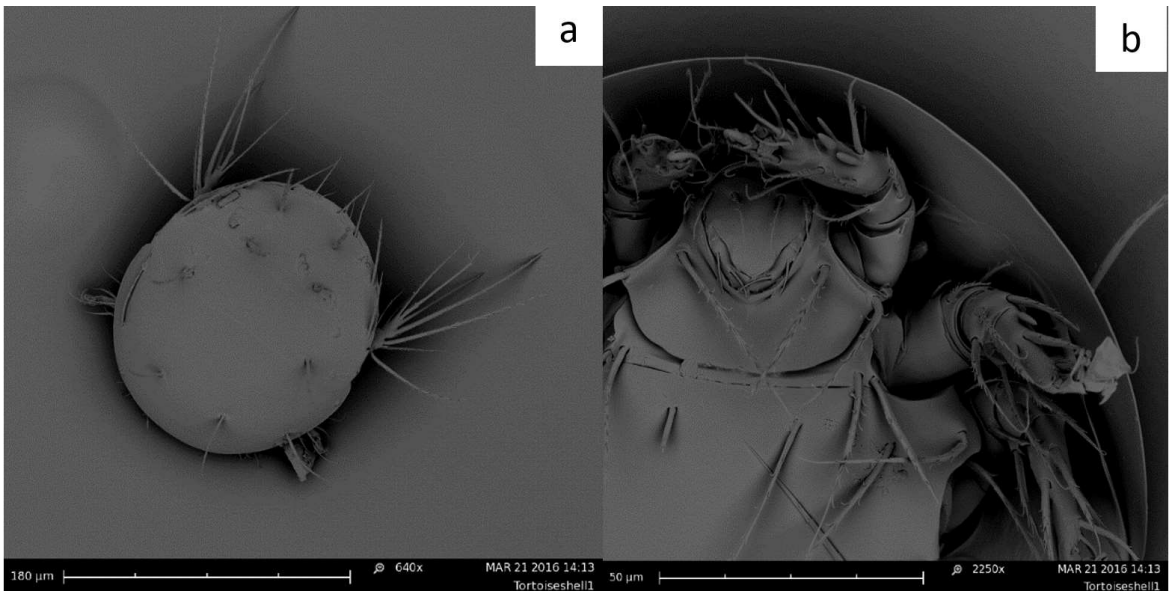


Fig 3.41. SEM images of scutacarid mite collected from *Sphecodes*. a) Dorsal view. b) Ventral view.

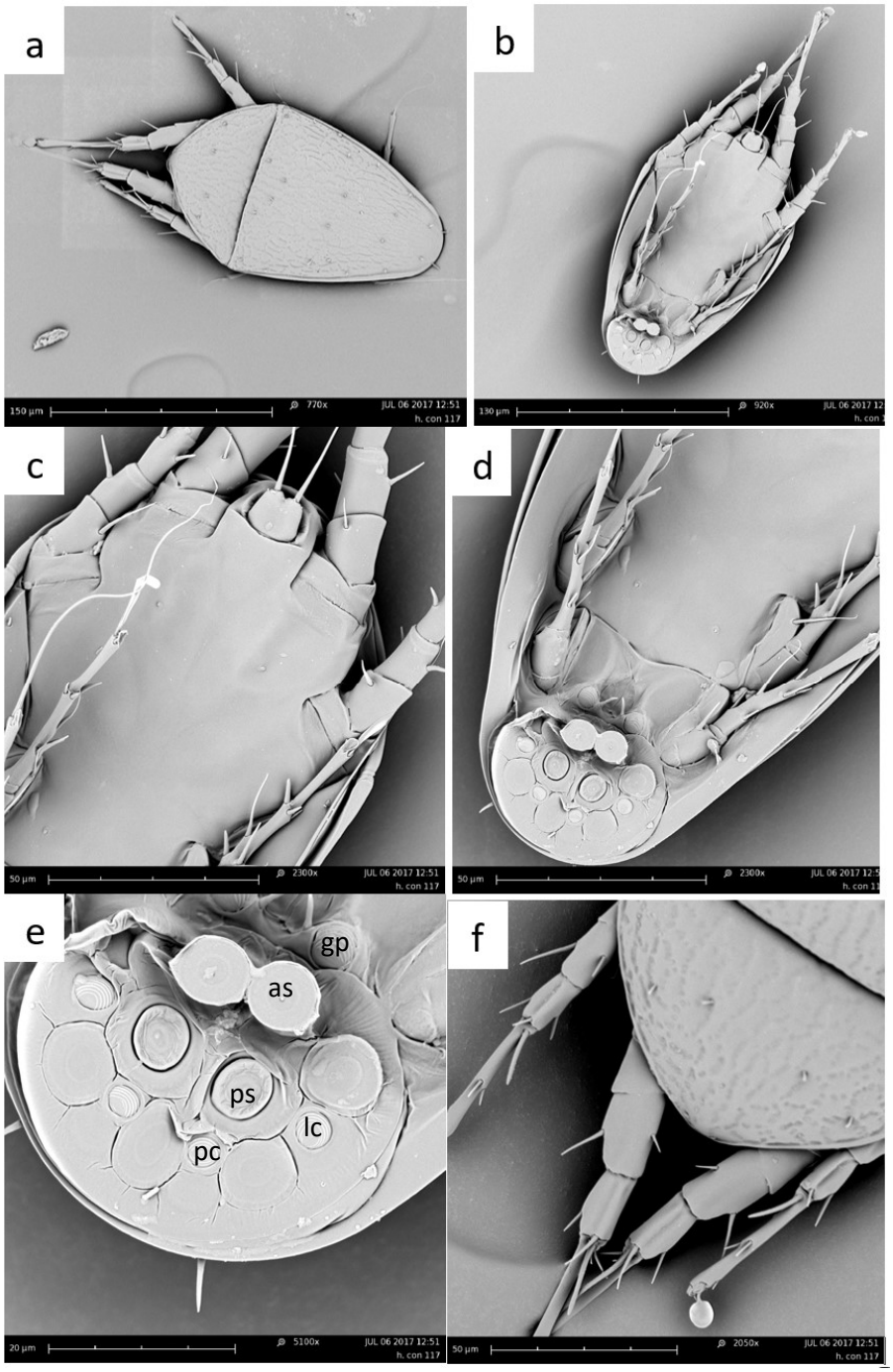


Fig 3.42. Unidentified histiostomatid mite, potentially a related *Anoetus* species, recovered from adult female halictid, tentatively identified as, *Halictus confusus* on the University of Saskatchewan campus in 2013. a) Dorsal view. b) Ventral view. c) Ventral view showing diminished cxI conoides. d) Ventral view showing diminished cxIII conoides, and presence of gp and suckerplate conoides. e) Caudal suckerplate, labels as assigned in Fig. 3.23. f) Dorsal view and texturing of integument.

It should be noted that other bees, located nearby and also belonging to *Halictus*, had their own different type of mite. A member of the halictid subgenus Seladonia, tentatively identified as *Halictus confusus*, nesting on campus (collected June 18 and July 11, 2013) also had a type of mite very similar in appearance to *Anoetus halictonida* (Fig 3.42a-f), with the most obvious differences being the lack of somatic conoides cxI and cxIII (Fig 3.42c,d) beyond those of the suckerplate (Fig 3.42e), dissimilar sculpturing on both of the dorsal shields (Fig 3.42a,f), and a narrower gnathosomal remnant (Fig 3.42c). Correspondence with Dr. H. Proctor, University of Alberta, to whom a prepared slide was sent, indicates that this mite would be a separate species, based on the lack of conoides.

3.4 Discussion

3.4.1 Mite distribution

The total number of mites (all deutonymphs) of *Anoetus halictonida* per adult bee of *Halictus rubicundus* was highly variable throughout the field season. This result is not unexpected as the specific ages of bees were unknown and a bee can only be sampled once, easily. It would not be unreasonable to assume that younger, unmated bees would show changes in their total mite loads as time passed, but this would require repeat sampling and was outside the parameters of this study. Whether the bees show any increase or decrease in total mite load would likely be dependent on the sex and age. The high variability in total mite load may reflect seasonal differences. The major peaks in total mite load in adult females in early May, late June, and early July may reflect the emergence of the new generations, the overwintering foundress females that construct the nests, and the resulting generations, respectively. The higher mite loads on adult female bees in late June and early July could also be related to the emergence of adult males, assuming the mites manage to transfer from adult male bees to adult female bees in large numbers. Mites do occasionally disembark, intentionally or not, onto petals while the bees visit flowers (section 3.3.6.1). Despite the firm attachment of each deutonymph to its bee host via the caudal suckerplate, some of the bee's original mite load will likely be lost due to factors such as host grooming, and the intentional movement of mites into the cells, and possibly to other individuals serving as new hosts.

Adult female bees had an average mite load of 53 deutonymphs, with most females (96.3%) having at least one. The large proportion of female bees having mites indicates that the

methods the mites use to transfer to the next generation are usually successful. On the contrary, almost half (42.9%) of the adult male bees lacked mites. It has been shown previously that mites do not discriminate between sexes and can develop in cells containing either sex (Eickwort 1994). The prevailing idea is that mites present on male bees are at a disadvantage as the male bees do not construct or provision nests, and male bees will be less effective at dispersing mites as the bees do not enter the nests where the next generation of mites will develop (Eickwort 1994). Those mites that lack access to the next generation also lack the opportunity to advance through the next stages and reproduce. Males were occasionally found within the nests during excavation, but these individuals were not shown to have any access to new cells. It is generally thought, based on observations of other species (Cooper 1954), that the mites on males will transfer during copulation with the female. There is no indication that this transfer occurs in the opposite direction, i.e., mites transferring from females to males. Based on this assumption, it would be expected that 42.9% of adult male bees that had no mites would either have mated (or at the very least, were close enough to a female to trigger the mites' departure) in the past, or had no mites to begin with. The latter situation seems unlikely, because only 13.6% (3 of 22) male pupae or pharate individuals, from this same population, lacked mites on their bodies during development. As there is no separation of different adult male and female bees based on age or mating history, these factors being unknown, this possibility cannot be proven. Age of adult bees can be a challenge to determine, and features that could indicate an older bee (i.e., blunted mandibles or tattered wings) are not reliable indicators of age alone.

Based on the very high proportion of female bees that harboured mites, the mites themselves are proficient at transferring. It is unknown if the mites possess the ability to discriminate between male and female bees already at the egg stage, but the mites appear to be opportunists. As the female bee closes off each brood cell within the nest, the egg is the stage at which the mites would need to be present to be sealed in the cell with the new bee, as *Anoetus halictonida* is not one of the mite species reported as being able to dig through soil, which has been reported from certain mite species in which the phoront is the adult female mite (Eickwort 1994). There were no mites observed on the eggs, although they were occasionally found on the cell wall lining. It should be noted that the range of the total number of mites on female bees was over twice that of the mite load on males, and the average mite load on females was much higher. This could indicate that there is a preference among the mites for female hosts, but if the mites

were unable to distinguish host sex then this disparity could be the result of size limits of the male bees, or perhaps the grooming behaviour of males may be more effective. The male bees were generally smaller than female bees (see section 2.3.4), and those areas on the male bee body utilized as attachment areas may be spatially restrictive. If there is a preference for female hosts, as is generally assumed, then the deutonymphs may have a preference for when they disembark, possibly early in the year, when the overwintering, fertilized foundress females that produce the female-biased first generation construct their nests. At any rate, the data from this study strongly suggest that there is transfer of mites of *A. halictonida* during development of male bees of *H. rubicundus*, the mean load per immature (pupae; pharate individuals) bee being 35.9 ± 41.3 (n=22), approximately double the number of deutonymphs (17.2 ± 25.6, n=28) on adult bees.

As female bees of *H. rubicundus* often lay multiple eggs (a single one per brood cell), it would be expected that only some of the deutonymphs disembark at any time, based on the fact the number of adult females of *A. halictonida* observed per cell never exceeded 11, and because such a high proportion of bees had any sized mite load. If all of the mites disembarked at once, there should be more bees with no mites. What triggers the departure is unknown, possibly chemical, but it could be as simple as the female bee brushing against the cell walls as she provisions and lays her egg. Compared to the total mite load of adult male bees where the majority (64.3%) of individuals had only 0-10 mites, and the higher mite densities had approximately equal numbers of representatives, the mite loads of adult female bees showed a gradual decline. This disparity could indicate that the separate sexes lose mites differently, the male hosts having large numbers of deserters when the opportunity arises at copulation, with the female hosts gradually losing small proportions of their mites as they forage, groom, and provision.

Whereas individual adult female *H. rubicundus* occasionally have very uneven loads, overall the majority of the bees analyzed showed symmetrical, non-significant distributions on their left and right wings. Similarly, all four of the mite species that Cross and Bohart (1969) examined on the bee *N. melanderi* appeared to be in a bilaterally symmetrical distribution, although certain areas had to be judged subjectively. However, not observed in previous studies on adult bees was a marked bias ($P<0.0005$) of deutonymphs of *A. halictonida* on the hindwings versus on forewings, despite the differences in wing size.

There was no apparent pattern for the total mite loads of immature bees over time. The mite loads of female pupae showed some similarities to adult female bees, such as the large ranges, 0-134 and 0-167, respectively, with very few female bees of any stage (adult, pharate, pupa) lacking mites altogether. Although sample sizes were slightly different, adult female bees had a larger average mite load [53.1 ± 44.6 (s.d.), (n=54)] as compared to immature females [32.6 ± 31.0 (s.d.), (n=40)], again suggestive that females receive deutonymphs via net transfer, when they have reached adulthood. At least half of female adults, pupae and pharate individuals had mite loads between at least 1 mite to around 60, whereas increasing mite loads were progressively rarer. Whether this is typical is unknown as the only other information on mite loads of *Anoetus halictonida* concerns a separate, unnamed at the time of publication, species of *Megalopta* (possibly *M. genalis*) (Halictinae: Augochlorini), which is distantly related to *H. rubicundus* (Halictinae: Halictini), but has a spatially separated range, having been collected in Central America (Engel and Fain 2003). The authors noted that 20% of the adults of the new species had mite loads that ranged from 1 to 72 per bee (sex not indicated). Moreover, both male and female *Halictus sexcinctus* were reported as hosting *Histiostoma halicticola* with 17 (77.3%) bees of the total (n=22) examined carrying 532 deutonymphs (Fain and Erteld 1998). Of the 6 female *H. sexcinctus*, 5 (83.3%) had mites, with ranges from 1-60. The average mite load for female *H. sexcinctus* was 29.6 ± 23.3 (s.d). Of the total male *H. sexcinctus* (n=16), 12 had mites (75.0%), and mite loads ranged from 2-95. The average mite load was 32.0 ± 36.1 (s.d.).

Among male *H. rubicundus* there were the occasional absences of mite loads of certain sizes in the middle of the distribution. This scarcity of intermediate mite loads could be due to sample size, but it should be noted that all of the male bee stages show an absence. In adult male bees, there are no mite loads of 40-50, in male pupae there are no mite loads between 40-100, and pharate male individuals lack mite loads of 40-80. Therefore, the male bees either had very low mite loads or very high mite loads. Again, there are fewer immature male bees without any mites (13.6%), in comparison to adult males (42.9%).

The caudal suckerplate *A. halictonida* has not been previously imaged using SEM. The original line drawing for the species description (Woodring 1973) was unable to show the flexibility that caudal suckerplates have. The two pairs of suckers and the wing-like margins of

the mantle of the suckerplate have a range of movement that single photos do not illustrate. How precisely the caudal suckerplate exerts suction is not known.

Other mites have been imaged by SEM, which provides a starting point for comparisons, but providing labelled photographs of *A. halictonida* is somewhat hampered by disagreements in terminology (see conoide vs disc vs conoidal seta)(apodeme vs cuticular sucker). A previous description of the caudal suckerplate of *Sancassania mycophagus* referred to parts of the caudal suckerplate (unlabelled, but present in Woodring's line drawing) as either lateral suckers or apodemes (2 lateral or the unpaired posterior). Currently, the term apodeme is an important characteristic used in keying mites and refers to something different, namely ingrowths of the exoskeleton. Other individuals refer to these "apodemes" as cuticular suckers (Klimov et al. 2004; Bee Mite ID online, University of Michigan).

The caudal suckerplate of *Anoetus halicticola* provides a comparison to the suckerplate of *Anoetus halictonida*, with the same basic arrangement (Fain and Erteld 1998). There are paired flexible anterior suckers, and paired sunken posterior suckers. There are the same number of cuticular suckers (2 paired anterior, 2 paired lateral, and 1 unpaired posterior), with conoides in between (4 in total on the suckerplate, 2 lateral conoides (**lc**) and 2 posterior conoides (**pc**)). However, many of these microstructures, plus the cuticular suckers (lccs, pccs) associated with the lateral and posterior conoides of the caudal suckerplate, were not identified by Woodring (1973) and hence are newly reported for *A. halictonida*. When these two species of *Anoetus* diverged is unknown, but the similarities in suckerplate morphology lend support to the idea that certain features of the deutonymph are conserved. Of all the *Anoetus* species described by Mahunka (1974), similarities are most obvious between *Anoetus halictonida* and *Anoetus orientalis* (Mahunka 1974). The caudal suckerplate of *A. orientalis* was drawn with two faint areas that may represent the pccs labelled previously (Fig 3.26), which does not appear in Woodring (1973)'s original line drawing. However, the shape of the gnathosomal remnant is more rounded and less square-shaped when comparing SEM of *A. halictonida* to line drawings by Mahunka, and the empodial claws of *A. orientalis* appear comparatively much larger than those of *A. halictonida*. It was noted by Dr. Klimov that despite the similarities, the setae on midtarsi III-IV are much shorter in *A. orientalis* than those of *A. halictonida*.

3.4.2 Mite location on the host body

Bees known to be associated closely with mites often have areas of the host body termed acarinaria. The term acarinaria is generally used to refer to certain anatomical features, like pouches or other concavities, that provide a secure attachment site for dispersing mites and are variably hypothesized to have evolved to retain mutualistic mites, or as a defense mechanism against harmful mites (Biani et al. 2009, Klimov et al. 2007). Cooper (1954) reports that the original use of the term was specifically used for the enclosed acarid chambers of xylocopid bees, but was later expanded to include integumental cavities of various location and construction, and describes male wasps (*Ancistrocerus antilope*) as having acarinaria. Eickwort (1994) described acarinaria as specialized pouches present on female bees that are used to carry phoretic mites and as evidence of coevolution. This term has also been used to describe a deep, setose furrow present on the second tergite of *Halictus sexcinctus*, which has been reported as a host of *Histiostoma halicticola* (now referred to as *Anoetus halicticola* in certain databases) in Germany (Fain and Erteld 1998). The same study noted the presence of mites in other body regions, such as the propodeum, but declined to utilize the term acarinaria in reference to them.

Halictus rubicundus do not necessarily have acarinaria, but they do have specific locations that serve as acarinaria. Whereas mites may be found in specific, small concavities in the sculptured exoskeleton, these, perhaps, should not be termed acarinaria. There are small concavities near the wing bases, but whether these are acarinaria depends somewhat on the definition, as they may be true acarinaria (based on the less specific defintions of acarinaria), or may simply be concavities where mites happen to reside. Many of the most well settled areas are places that do not appear to have any sort of modification or specialization that would make them more attractive to mites. The wings, especially near the main veins, provide long furrows that offer a semi-temporary shelter (i.e., when the wings are folded), but would likely not be considered acarinaria. Adult bees of each sex have their own separate locations where mites are found most often. In adult females, the deutonymphs are usually found on the wings, specifically the lower surface, and dorsally at the junction of the mesosoma and metasoma. Many of the locations are difficult for the host to reach when auto-grooming, a characteristic noted previously with phoretic mites of many species being reported on their associated hosts in numerous places, such as between the coxae, on the metasomal sterna, at the junction of head and thorax, the

junction of thorax and metasoma, in intersegmental spaces, the genital chamber, at the wing bases, and on the wings (Eickwort 1994). It is unknown whether the mites intentionally migrate or congregate in these areas, or whether mites lack any preference and only those mites that attached in the protected areas remained when the bee groomed. The dorsal areas are generally out of reach of the legs when grooming, whereas the wings are often folded when grooming, and the scraping motion of the legs during grooming is not enough to dislodge mites on the opposite side of the wing. In addition, the veins on the wings form natural furrows that may serve as unintended protective areas. This extra protection could explain why mites are often found along the main wing vein. The upper surfaces of the wings of adult females are less populated by deutonymphs than the lower surfaces, with the exception of the jugal lobe. The jugal lobe has no veins and lacks resistance, sometimes folding over when the wings are at rest. In the *Megalopta* species previously noted as hosting *A. halictonida*, the mites were reportedly found at the extreme wing bases and on the first metasomal tergum, with no other regions mentioned (Engel and Fain 2003). Eickwort (cited from Woodring 1973) reported that once the adult bee ecdyses, the deutonymphs attach preferentially to the undersurface of the front wings. Deutonymphs of *A. halictonida* were often located on the undersurface of the wings (Fig 3.12), but data from this study indicates that the hindwings had significantly more mites despite decreased surface area. In addition, male adult *H. rubicundus* generally had deutonymphs of *A. halictonida* located elsewhere, the wings not appearing to be heavily populated (Fig 3.13. 3.14). However, as the age of the adult bees was not determined, this disparity could reflect an unseen transfer of the mites from the wings of the male *H. rubicundus* to the adult females during copulation.

The deutonymphs of *A. halictonida* on adult males of *H. rubicundus* are often located ventrally, primarily occupying the underside of the head, between coxae, and on the basal leg segments. The wings - both the lower and upper surfaces - had much lower mite frequencies than adult female bees. The difference in mite location between male and female adult bees indicates that mites can distinguish between the sexes and orientate themselves based on that determination, possibly with the specific purpose of transferring from male to female bees. Differential attachment location based on species of mites and sex of bee host has been noted previously, as in *Caloglyphus boharti* and *Glyphanoetus nomiensis*, two phoretic associates of the alkali bee (*Nomia melanderi*) which possess the modified deutonymph stage that acts as phoront (Cross and Bohart 1969). Deutonymphs of *C. boharti* were found almost exclusively on the

metasomal segments, in the intersegmental spaces of *Nomia melanderi*, likely to be found ventrally on males, but dorsally or ventrally on females. Male *N. melanderi* were more often infested and carried higher mite *C. boharti* burdens, on average. The authors hypothesized this to be a direct consequence of the deutonymphs of *C. boharti* being comparatively more active, leaving the cells to find new hosts and likely attaching to the first bees to emerge, which are the male *N. melanderi*. In comparison, *G. nomiensis* attached to the upper surface of all four wings, but there were often large groups of deutonymphs in the lateral depressions of the modified fifth sternite of males. Male *N. melanderi* appeared to have heavier burdens of *G. nomiensis* mites than females. The deutonymphs were generally attached in roughly equal numbers on each side of *N. melanderi*, occasionally asymmetrically, but usually displaying a bilateral symmetry that may be involved in minimizing aerodynamic interference by not upsetting the balance of the bee body (Cross and Bohart 1969). Adult females of the scutacarid mite, *Imparipes americanus*, are also associates of adult *N. melanderi*, apparently infesting male and female bees equally, but with a notable distinction being that male bees lose mites at a faster rate. Cooper (1954) reported that *Ancistrocerus antelope* possessing heavily asymmetrical loads of deutonymphs of *Ensliniella trisetosa*, it was very likely an indication of a recent loss that would soon be rectified by the redistribution of deutonymphs from the propodeal acarinarium.

The comparable phoretic association between *H. sexcinctus* and *A. halicticola* also has some differences in deutonymph location between male and female bees (Fain and Erteld 1998). Deutonymphs on females were primarily located on the second tergite, the location of the furrow identified as the acarinarium, as well as the propodeum (lateral and dorsal), the hypoepimeral fields, and on the ventral thorax. It should be indicated that beyond indicating that the acarinarium appeared to be the preferred location, the mite loads per adult were not further divided into body region. The males primarily had their mites located ventrally on the thorax, with the exception of one adult male bee which had at least some of its mite load distributed on its second tergite.

In certain locations on the host bee body where the mite density is high, the positioning of mites is slightly different. Individual mites, when distant from conspecifics will lay flat against the host body surface. Mites in more crowded areas will occasionally slightly overlap, with the caudal suckerplate firmly attached to the host body, but the anterior end slightly raised. In places

with very high mite density, this orientation and positioning of the deutonymphs is even more exaggerated (e.g., Fig 3.19b compared to Fig 3.20b and Fig 3.21c), and the mites have their anterior legs raised. The mites' body positioning is not unlike the "questing" position described in other arachnids (honey bee tracheal mites, *Acarapis woodi*, [Sammataro and Avitabile 2011] and ticks). This positioning could reflect the high density of mites in a restricted location, allowing conspecifics to maintain a secure point of contact with the host, while maximizing the opportunity for a relatively rapid transfer by a number of mites confined to a particular area. These high density areas may be desirable to the mites for some reason, leading to overcrowding. These areas may be desirable for reasons like superior shelter, or as a better place to disembark.

The positioning may also improve successful transfer of mites. If a mite is trying to transfer from a male to a female, the questing position could improve sensory capabilities, or just get the mite closer to its destination. Those mites that are near the cluster margins may be less protected, but could have an advantage when trying to transfer, although how much time is needed to move between and off bees is unknown. It was noted that the deutonymphs, specifically deutonymphs removed from a very young adult attempting to remove its exuvium, are capable of relatively quick movement as compared to mites removed from adult bees, which are somewhat sluggish.

Immature bees had very little difference in mite location between the sexes. Both male and female immature bees had mites located all over their bodies. There were areas, especially on the mesosoma, that were highly utilized by mites on both sexes of *H. rubicundus*, but the immatures differed greatly from the adults in which locations are heavily populated by mites. When the bee pupae mature into adults, the mites will either have to migrate to the new areas, or be left behind on the exuvium or brood cell wall. The immature bees lack the range of movement of adult bees, and the mites are in less danger of being removed, such as by grooming. Even if the mites do fall off, the pupae are restricted from moving too far by the cell walls. The areas on the pupae where mites are located may have alternative advantages, such as a preferable humidity or a more plentiful food source. Previously, Eickwort (unpublished observations material collected in 1969, cited from Woodring 1973) noted when the protonymphs first moved onto the bee they were especially abundant ventrally on the head and thorax, whereas older deutonymphs were found clustering on the sides and dorsum of the pupal propodeum and metanotum. This

clustering was also observed in the pupae excavated from site 1 during 2015 (Fig 3.15, 3.17), but there was no attempt to separate younger deutonymphs from older deutonymphs. In addition, deutonymphs were found clustering on the wings of pupae (Fig 3.16, 3.18), but not especially concentrated ventrally on the head or thorax, instead appearing scattered in distribution on both sexes (Fig 3.15, 3.17). The mites were often found in areas that were not groomed, but observations of pupal bees indicated that the mites actively migrated to these areas, as opposed to attaching randomly (Eickwort 1994).

3.4.3 Mite stages

The deutonymphs, as compared to the other stages present, are comparatively simple to identify, being heteromorphic and heavily modified to properly serve as hypopi. There is a well acknowledged lack of keys for mites based on stages other than the deutonymph. For example, the original paper describing *Histiostoma halictonida* lacked a description of stages other than the adult female and deutonymph (Woodring 1973). Based on Eickwort's notes (unpublished, made on material collected in 1969), those mite stages found during the excavation of cells with larval stage bees present are either tritonymphs, or adults. The gravid adult females are easily identified under transmitted light microscopy, due to the presence of eggs internally. Previous studies of related histiostomatids indicate that there are few features that distinguish tritonymphs from adults, complicated by the fragile state of the females. Some of the mites pictured appear to have only six legs, which would immediately indicate they are larvae, but this could be an effect of the way they were prepared. According to Eickwort's description in Woodring (1973), around the time when the bee larva defecates tends to coincide with the period when gravid adult females were present. It is unknown how much variation there is in generation time, or if multiple generations can grow to maturity under optimal conditions. Size is not particularly useful in determining a mite's phenological stage due to variation (Fig 3.1f) based on a description of *Histiostoma julorum,* their host not further specified beyond being flies that visit decaying organic matter (Hughes and Jackson 1958); however, the large adult females were at least twice the size of the presumed tritonymphs.

The mite stages observed in this present study appear to completely lack mouthparts capable of directly harming the larvae, although if parasitism does occur it could happen through non-oral means, through an alternative orifice or if the cuticle is porous. The deutonymphs are by

definition hypopi that lack mouthparts and associated functional feeding structures, whereas the presumed tritonymphs had a scoop-like apparatus with rounded edges, and two long, flexible structures in the centre. What may have been feeding behaviour was observed only once in a mite photographed with SEM (Fig 3.37b), with its anterior end partially buried in the debris on the larval bee integument.

Certain exceptions to the non-feeding characteristic associated with deutonymphs have been observed, despite a lack of mouthparts or functional gut. Radiolabelling was used to show that deutonymphs of *Hemisarcoptes cooremani* can potentially acquire materials (specifically water) from its host, *Chilocorus cacti* (Houck and Cohen 1995), despite being previously classified as a phoront and maintaining the heteromorphic morphology of a hypopus. It was hypothesized that water is acquired via the action of the caudal ventral suckers on the caudal suckerplate, in addition to water vapour being absorbed across the cuticle (Houck 1994). Another example of non-oral parasitism is displayed by the parasitic deutonymphs of the hypoderatid mites, *Neottialges evansi*, which lack mouthparts and a functional foregut, in addition to having a midgut and hindgut that appear incapable of processing food (Alberti et al. 2016). The parasites are present in the subcutaneous layer of their hosts and it is believed that they become engorged through the use of two pairs of genital papillae that could be nutrient-intake organs allowing for the absorption of liquid materials. Observations on deutonymphs of the mite *Hemisarcoptes cooremani* show a behaviour which could create a negative pressure through use of its modified third and fourth pairs of legs acting as levers to raise and lower the suckerplate while it is attached to the elytron of the bee, which could explain how the mite attains water from its host (Houck 1994), but could also provide some insight into how these mites attach to their hosts.

The adult females observed were less than 500 μm long (Fig. 3.35) as compared to the original description of the species by Woodring (1973), who reported the average length of 5 females to be 650 μm. It is possible that this disparity is a result of intraspecific variation, much like the variation in life cycle stages of *H. julorum* (Fig 3.1f from Hughes and Jackson 1958).

3.4.4 Mite weights and phoretic loads

The pooling of mites, specifically deutonymphs, to determine individual weights was not ideal. However, even the combined weights of multiple mites were still almost negligible and indicates that although bees may carry upwards of 100 mites, the mite load is unlikely to affect

the bee unless the mites irritate their host or are located in body areas that restrict bee movement, possibly at the junctions of leg segments, or at the wing bases. Even in the adult female bee with the highest mite load (167 deutonymphs), which represents approximately 93 micrograms, the mite load is still well below 1% of the bee's weight. The majority of adult bees, both male and female, had much lower mite loads.

Evidently there are no reported weights of pollen loads for *H. rubicundus* available; however, adult females of *Halictus medionitens* have been reported to carry fresh pollen loads (*Rubus deliciosus*) with a mean weight of 1.65 mg ± 1.35 (s.e.), a pollen load that is 18% of their body weight (based on data calculated from Clements and Long 1923; see Davis 1997). This finding indicates that even the highest mite loads of *A. halictonida* on *H. rubicundus* are negligible, assuming that the pollen loads for *Halictus* species are comparable.

Woodring (1973) reported the average length of 5 deutonymphs at 250 µm, which is much larger than the average of 190.3 µm ± 12.7 (s.d.) reported here. The lengths of *A. halictonida* deutonymphs were generally around 1.5 times their respective widths. It could be a regional difference, a nutritional one, or it could be that deutonymphs from different body regions of the host bee (i.e. the wings) are different sizes. For comparison, the deutonymphs reported by Mahunka (1974) had longer and wider deutonymphs. *Anoetus ligulotrichus* had lengths of 218-232 µm and widths of 121-134 µm. *Anoetus orientalis* deutonymphs, which was the most morphologically similar of the mites described by Mahunka, had lengths of 229-237 µm and widths of 149-158 µm. More similar to what was found in this study, *Anoetus szelenyii* deutonymphs were 193-202 µm in length and 141-147 µm in width, which is still much wider than an average width of 119.5 µm ± 8.8 (s.d.) found for *A. halictonida* deutonymphs.

3.4.5 Transfer of mites

Previous work described in section 3.3.6.1 showed that mites did occasionally drop off, or intentionally disembark, from their hosts onto flowers while the bee foraged. The attempts to investigate whether mites could be induced to transfer between individual bees were unsuccessful. Collection of dead bees showed that deutonymphs will sometimes remain on their dead host despite there being no opportunity to transfer. This prolonged residency could indicate that these deutonymphs are heavily reliant on their host. It has been reported that mites can survive in the absence of a living host. Mites were observed feeding on the feces of a dead pupa

which had been removed, although the mite stages involved were not mentioned (Eickwort cited from Woodring 1973).

Since *A. halictonida* has been found on a separate species of *Megalopta* (Engel and Fain 2003), the anoetid mites have transferred at some point in the past and may freely transfer between species of Halictinae. The ranges of these two bee hosts are spatially separated, with the holarctic *H. rubicundus* found as far south as California, Arizona, and New Mexico and the *Megalopta* species only found in Costa Rica and Panama. Activity of these two bee taxa is also temporally separated, as *H. rubicundus* is diurnal and *Megalopta* is nocturnal (Engel and Fain 2003). The authors suggested that such a transfer could be achieved through use of an intermediary, such as *H. ligatus*, assuming that the ranges of the involved halictids overlapped.

3.4.6 Comparison to other mites on halictid bees

Anoetus halictonida was not the sole histiostomatid found on the University of Saskatchewan campus and a few comparisons were made to determine if the mite of interest showed any sign of transferring between species. Some adult females of what is presumed to be *Halictus confusus* collected in a previous year (2013) were given greater scrutiny, but their mite associates were easily distinguishable from those of *A. halictonida*. These mites showed some similarities to *Anoetus ligulotrichus,* associated with *Halictus fulvocinctus*, and *Anoetus tunisiensis*, associated with an unnamed *Halictus* species (Mahunka 1974). Both of the aforementioned anoetids have a narrowed gnathosomal remnant and lack conoides cxI and cxIII. Of the two, *A. tunisiensis* has the conoidal remnants more linearly aligned and a filamentous distal tarsal seta on leg III, similar to the unidentified mite, although it is unknown if these characteristics are important for a postive identification. There were also *Sphecodes* that showed an interest in the *H. rubicundus* nests, and carried at least two species of mite. One was a type of scutacarid, possibly an *Imparipes* sp., the other being nearly indistinguishable from *A. halictonida* except under SEM (see section 3.3.7, Fig. 3.42). It must be considered that these differences may be due to intraspecific variation.

CHAPTER 4 - CONCLUSION

4.1 Summary

This study of an urban population of *Halictus rubicundus* [Christ] (Hymenoptera: Halictidae) has demonstrated a high frequency of individuals with *Anoetus halictonida* [Woodring] (Sarcoptiformes: Histiostomatidae) mites present both on bees and within brood cells of the nests. The mites are located differentially on adult bees based on sex, which is sometimes posited to result from venereal transmission of mites (Cross and Bohart 1969), although direct observations of venereal transfers in hymenopterans are lacking. Individual bees can host high densities of mites with little to no harm observed. Considering the lack of observed engorgement, the complete lack of functional mouthparts and gut of deutonymphs reported in other mite species by OConnor (1982), as well as there being no negative effects attributable to the mites, there is no evidence for parasitism. It is possible for phoretic organisms to become covertly parasitic on their hosts despite a lack of bodyparts that would otherwise prevent feeding (e.g., *Hemisarcoptes*; Houck 1994). However, whereas certain mites are capable of taking advantage of their hosts non-orally for the purposes of hydration and possibly nutrition, it should be noted that the two examples noted previously have some very particular systems that allow these deutonymphs to alter their symbiotic relationship. Much more likely is a mutualistic relationship (see Biani et al. 2009), in which *Laelaspoides* mites perform a role in sanitation. The relationship appears to be mostly exclusive, with no other mites collected from the bodies or within nests of *H. rubicundus*, and the only other host of *A. halictonida* reported in the literature being *Megalopta* sp. in Costa Rica and Panama (Engel and Fain 2003). *Anoetus halictonida* may be a successful competitor for other invading mites that could attempt to feed on the provisions or immatures within a cell, but are outcompeted by the phoronts that have adapted to their halictid hosts. Incidentally, scanning electron microscopy of *A. halictonida* occasionally revealed a small number of pollen grains on the mite body, but there was no evidence of any phoront externally on the mite (i.e., an apparent lack of hyperphoresy in *A. halictonida* in this population of *H. rubicundus*).

It is possible that the specific positioning of *A. halictonida* deutonymphs on adults of *H. rubicundus* is not entirely due to their removal by auto-grooming, which is the most frequently encountered explanation for why mites choose specific body locations on their hosts. Whether the mites can actually navigate to a favourable position instead of happening to attach and not being groomed off has not been well demonstrated, but these deutonymphs can turn and do tend to orientate in an orderly fashion. Interestingly, deutonymphs of *Ensliniella trisetosa* on adult male *Ancistrocerus antilope* have been reported redistributing themselves (Cooper 1954). The adult male wasps have lateral acarinaria, one left and one right. When all the mites are removed from one lateral acarinarium and the male wasp isolated, the mite loads on the two lateral acarinaria will be approximately equal within a day.

Weighing with a microscale was originally used in hopes of determining whether mites from different locations showed any disparity in mass, but the necessity of pooling reduced the value of such a measure. For example, in adult female bees, the underside of the wings seems to be a place of highest mite frequency and density. The wings, while difficult to groom, may have other advantages, such as being easier to remain attached or may allow better access to the physiological cues that indicate information about the host. This second hypothesis loses some support when considering that the wings were the only body location where the mites remained on bee cadavers collected. The compact, overlapping (shingle) arrangement of mites in high density areas may serve a purpose beyond space-saving. It has been suggested (Cross and Bohart 1969) that mites disembark in blocks, which could be related to the mites' particular sites of congregation. With the exception of a few *H. rubicundus* with very noticeably left or right biased loads, the majority of adult female bees appeared to have roughly symmetrical numbers of mites on their wings. If deutonymphs of *A. halictonida* do disembark in groupings, this could explain the occasional asymmetrical mite load, with a large cluster from only one side getting off the host at approximately the same time. Even if the mite loads are comparatively tiny (less than 1% of the bee's weight), the bee may still be aware of the sudden disparity in weight and groom to regain balance or mites located in other body regions may migrate to the newly vacated spot.

Attachment of deutonymphs to their host, even at high-density locations, is attributable to their caudal suckerplates. The suckerplate has a range of motion that can be inferred through many of the SEM photos. The exact ways the different parts of the suckerplate cooperate to

attach the deutonymph can be difficult to determine, since these parts are usually obscured. The conoides were suggested as being releasing organs (Fain and Erteld 1998), but appear striated. They may serve an additional purpose in providing traction, much like tire treads, possibly against grooming by the host or excess surface moisture on the host. The structure of the posterior suckers is noticeably different from that of the anterior suckers. In several images, the posterior suckers appear deflated, and are possibly capable of exerting a negative pressure by doing so, thereby more firmly attaching the mite to the surface.

In comparison to deutonymphs on other hosts, at least some aspects of the *A. halictonida* deutonymph are conserved, such as dorsal-ventral flattening and the comparatively small range (Fig 3.1f) of deutonymph lengths (Hughes and Jackson 1958). It may be that since the deutonymph stage is so critical to the continuation of its respective species, variation is selected against. Several mite species known to be phoretic on Hymenoptera, such as the anoetid found on what is presumed to be *H. confusus*, have similar caudal suckerplates, but this likeness could be a result of recent speciation. As mentioned previously, the anoetids on *H. confusus* lack conoides, with the exception of the two pairs on the caudal suckerplate, possibly indicating that the conoides on the suckerplate are much more important, or perhaps more evidence that the structure of the caudal suckerplate is strictly conserved. Certain aspects of the caudal suckerplate morphology are similar to more distantly related mites, specifically the number and arrangement of suckers and conoides, even if the overall appearance is somewhat different.

4.2 Potential future research

Some of the literature on histiostomatid mites has become outdated and requires some official recognition of Mahunka's reclassification. There is a lack of information on other stages, such as the tritonymphs, of the life cycle of *Anoetus halictonida*, which would be valuable to augment the mite to the same level of the description as *Histiostoma julorum* (Fig 3.1) by Hughes and Jackson (1958). The paper by Woodring (1973) first describing *Histiostoma halictonida* included line drawings of the adult female and deutonymph, but there are no descriptions or keys for the other sex (if present at all), or other immature stages. There is little information as to how *A. halictonida* behaves. How do the deutonymphs know when to disembark? Is there a mechanical cue (as indicated previously with *H. julorum*), or is there a chemical component? In addition, how do the mites know when it is not a good time to disembark? It is rare to find an

adult female of *H. rubicundus* without mites of *A. halictonida*, so she either loses mites gradually, or she loses many at once and has frequent enough contact with other conspecific bees that she has a near constant mite load, which seems unlikely.

Several of the histiostomatid mites are difficult to distinguish at their deutonymph stages, the stage for which most keys are based, and it may be that there are unidentified cryptic species, requiring molecular DNA-based study to differentiate. Other mite species were found on other adult halictid bees (e.g., *Halictus confusus*) in the area, but on separate hosts (i.e., *Lasioglossum*) and in far fewer numbers. How common is it for cleptoparasites like *Sphecodes* to act as vehicles for transmission of otherwise exclusive mites to new species, and are there other more common ways for mites to transfer other than on flowers or inflorescences?

A better understanding of the factors governing choice of ground-nesting sites by *H. rubicundus* may entice future aggregations to colonize areas and contribute to local pollination, and will allow greater conservation of this species in urban areas. In addition, where precisely members of this population of *H. rubicundus* overwinter was never determined, and there are varying accounts in the literature stating where this species overwinters. Efforts to investigate further were somewhat hampered by the ground freezing, so it would be interesting to determine more about this particular bee's methods in dealing with the cold.

Some data regarding the pollen collected by *H. rubicundus* was recorded from nest provisions, but actual flower species visited were not determined. This study has provided important information about pollen types collected by this halictid species, which will complement future surveys of flora visited by foragers of *H. rubicundus* in Saskatchewan. It would also be valuable to determine the pollination effectiveness of *H. rubicundus*, such as the importance of its role in early-flowering fruit species of Rosaceae in Saskatchewan. Future attempts to measure the average mass of pollen loads carried into the nest will provide estimates of the effort required by an adult female to furnish the provision for a single offspring. Also, a study to distinguish flower species visited by *H. rubicundus* for pollen collection and those visited for nectar collection, would be valuable for conservation purposes in urban settings.

Other insect parasites and parasitoids were present at the nests of *H. rubicundus*, such as some dipterans, but were not given an abundance of attention. The identification of cleptoparasites, as well as determining if they affect the dietary choices of *H. rubicundus* in the

same way cleptoparasites like *Sapyga* appear to affect *Osmia* (Spear et al. 2016), could provide insight into the shift from homogeneous pollen provisions to heterogeneous as the season progresses. It is currently unknown whether *A. halictonida* can transfer or will even attach to dipteran hosts, especially as several of the parasitic dipterans emerge from within the body of the host bee, possibly complicating the transfer of mites to the point of impossibility.

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Appendices

Legend: L-left, r-right, w-wing, prop-propodeum, ante.-antenna, thrx-thorax, f-fore, h-hind, t#-tergite#, s#-sternite#

Appendix Table 1. University collection of solitary and socially primitive bees

Family	Species	Collector	Date	Place	Mites Present	Distribution and Quantity	Bee Sex	Notes
Andrenidae	?	Kenneth M. King	7 8 1925	Holdfast SK.	N		M	
	?	Kenneth M. King	7 8 1925	Penzance SK.	N		M	
	?	Kenneth M. King	17 v 1925	Saskatoon SK.	N		M	
	?	?	12 iv 1918	Saskatoon SK.	N		M	Damaged antennae
	?	Kenneth M. King	May 12th 1925	Saskatoon SK.	N		M	
	?	Smith	4 vi 45	N.W.T.	N		M	
	?	Kenneth M. King	17 v 1925	Saskatoon SK.	N		M	
	?	?	13 iv 1915	Saskatoon SK.	N		F	Halictidae?
	?	Kenneth M. King	7 8 1925	Penzance SK.	N		M	
	?	NJ Atkinson	11 vii 1926	Tangle-Flags (2) SK.	N		M	
	?	?	28 May. 06	Lumsden	N		F	Halictidae?
	?	?	13 iv 1915	Saskatoon SK.	N		M	Damaged anntenna
	?	Kenneth M. King	10 viii 1925	Kennedy SK.	N		M	
	?	Kenneth M. King	10 viii 1925	Kennedy SK.	N		M	
	?	?	12 iv 1918	Saskatoon SK.	N		M	Dark brown, no pubsecence
	?	AE Cameron	29 iv 1918	Saskatoon SK.	N		M	Damaged antenna

	?	Smith	4 vi 1946	N.W.T.	N		F	Damaged antennae, pollen load present
	?	B. Fuller	8 vi 1944	Rockglen SK.	N		F	
	?	?	9 vii 1915	Saskatoon SK.	N		F	Damaged antenna
	?	S.M.	June 1940	Macklin Sk.	N		F	
	?	AE Cameron	29 iv 1918	Saskatoon SK.	N		M	
	?	?	31 v 1918	Saskatoon SK.	N		F	White pubescence, some pollen
	?	?	7/2/41	S'toon	N		F	Damaged antennae and thorax
	?	AR Brooks	25 v 1950	Saskatoon SK.	N		M	Damaged antenna
	?	?	13 iv 1915	Saskatoon SK.	N		M	
	?	AR Brooks	25 v 1950	Saskatoon SK.	N		F	
	?	?	26 vi 1919	Hirsch?	N		F	
	?	AR Brooks	25 v 1950	Saskatoon SK.	N		M	
	?	AR Brooks	25 v 1950	Saskatoon SK.	N		F	
	?	?	June 10th 1939	Brightsand L.	N		F	Damaged antenna
	?	?	11 viii 10	Indian Head	N		F	Some pollen present
	?	?	12 iv 1918	Saskatoon SK.	N		M	Damaged antenna
	?	AR Brooks	25 v 1950	Saskatoon SK.	N		M	
	?	Kenneth M. King	4 8 1925	Penzance SK.	N		M	Different family?
	?	JG Rempel	May 17th 1938	P.A. Park SK.	N		F	
	?	AR Brooks	25 v 1950	Saskatoon SK.	N		F	Damaged antenna
	?	AR Brooks	25 v 1950	Saskatoon SK.	N		F	

	?	AR Brooks	25 v 1950	Saskatoon SK.	N		F	Damaged left antenna
	?	JE McFarlane	August 1940	Waskesiu	N		F	
	?	?	20 iv 1918	Saskatoon SK.	N		F	Damaged right antenna
	?	AR Brooks	25 v 1950	Saskatoon SK.	N		F	
	?	?	12 iv 1918	Saskatoon SK.	N		M	
	?	AR Brooks	25 v 1950	Saskatoon SK.	N		F	
	?	AR Brooks	25 v 1950	Saskatoon SK.	N		F	
	?	?	12 v 47	Saskatoon SK.	N		M	Damaged antennae
	?	AR Brooks	25 v 1950	Saskatoon SK.	N		F	
	?	AR Brooks	25 v 1950	Saskatoon SK.	N		F	
	?	AR Brooks	25 v 1950	Saskatoon SK.	N		F	
	?	AR Brooks	25 v 1950	Saskatoon SK.	N		F	
	?	Kenneth M. King	May 17th 1925	Saskatoon SK.	N		F	
	?	Kenneth M. King	4 8 1925	Penzance SK.	N		M	
	?	NJ Atkinson	19 iv 1926	Saskatoon SK.	N		M	
	?	AR Brooks	25 v 1950	Saskatoon SK.	N		F	
	?	Kenneth M. King	17 v 1925	Saskatoon SK.	N		M	
	?	Kenneth M. King	May 14th 1925	Saskatoon SK.	N		F	
	?	Kenneth M. King	10 viii 1925	Kennedy SK.	N		M	
	?	?	5 vii 1947	Resolution	N		M	
	?	AR Brooks	25 v 1950	Saskatoon SK.	N		F	Damaged right antenna (tip)
	?	AE Cameron	29 iv 1919	Saskatoon SK.	N		M	

	?	?	Aug 9 19-8	S'toon SK.	N		F	
	?	JS Thompson	July 5th 1938	?	N		F	
	?	JS Thompson	June ? 1938	Waskesiu SK.	N		F	Damaged antennae
	?	NJ Atkinson	24 v 1925	Saskatoon SK.	N		F	
	?	?	May 28th 19-8	Saskatoon SK.	N		F	
	?	?	May 24th 19-8	Saskatoon SK.	N		F	Damaged antenna
	?	?	17 v 1916	Saskatoon SK.	N		M	
	?	AR Brooks	25 v 1950	Saskatoon SK.	N		F	
	?	?	May 28th 19-8	?	N		M	
	?	JST?	May 7th 1940	S'toon SK.	N		M	
	?	E Sandercock	21 vi 45	Indian Head	N		F	
	?	?	20 v 30	S'K'toon	N		M	
	?	B.Fuller	8 vi 44	Rockglen SK.	N		F	
	?	AR Brooks	25 v 1950	Saskatoon SK.	N		F	
	?	Kenneth M. King	4 8 1925	Penzance SK.	N		F	Halictidae?
	?	JW Joyce	July 28th 1940	Saskatoon SK.	N		F	Large pollen load
	?	JE McFarlane	Aug. 1St 1949	Waskesiu SK	N		F	
	?	AR Brooks	25 v 1950	Saskatoon SK.	N		F	
	?	?	15 vi 1915	Saskatoon SK.	N		F	Antennae damaged
	?	JS Thompson	July 21st 19--	SK.	N		F	Damaged antennae

	?	?	20 iv 1918	Saskatoon SK.	N		M	Damaged left antenna
	?	?	29 vi 1918	Saskatoon SK.	N		F	Extended sting, different family?
	?	?	4 vi 1911	Saskatoon SK.	N		M	Damaged antennae
	?	?	21 vi 1915	Saskatoon SK.	N		F	Damaged antennae
	?	AR Brooks	25 v 1950	Saskatoon SK.	N		M	Thorax, parasitic beetle larvae?
	?	JS Thompson	July 5th 19--	Waskesiu	Y	(2 right thorax: 1 loose,1 att.)(3 at pr-met junc.)	F	
	?	E Sandercock	7 ix 1945	Indian Head	Y	(1 at base right first leg)	M	
	Andrena sp.	L. Konotopetz	8 v 1950	Saskatoon SK.	N		M	*Cogswellia* flowers, damaged antennae
	Andrena sp.	AR Brooks	7 v 1950	Saskatoon SK.	N		M	*Salix* catkins
	Andrena sp.	AR Brooks	7 v 1950	Saskatoon SK.	N		M	Flowers of *Pulsatilla*
	Andrena sp.	AR Brooks	10 v 1950	Saskatoon SK.	N		M	Damaged left antenna
	Andrena sp.	LA Konotopetz	25 v 1950	White Fox	N		M	
	Andrena sp.	AR Brooks	12 v 1950	Saskatoon SK.	N		M	Damaged left antenna
	Andrena sp.	AR Brooks	12 v 1950	Saskatoon SK.	N		M	
	Andrena sp.	AR Brooks	12 v 1950	Saskatoon SK.	N		M	
	Andrena sp.	AR Brooks	12 v 1950	Saskatoon SK.	N		M	
	Andrena sp.	?	28/5/38	Saskatoon SK.	N		F	
	Andrena sp.	AR Brooks	8 v 1950	Saskatoon SK.	N		M	*Cogswellia* flowers

	Andrena sp.	L Konotopetz	8 v 1950	Saskatoon SK.	N		M	*Cogswellia* flowers
	Andrena sp.	AR Brooks	7 v 1950	Saskatoon SK.	N		M	*Salix* catkins
	Andrena sp.	AR Brooks	7 v 1950	Saskatoon SK.	N		M	Flowers of *Pulsatilla*
	Andrena sp.	AR Brooks	10 v 1950	Saskatoon SK.	N		M	*Salix* catkins
	Andrena sp.	AR Brooks	7 v 1950	Saskatoon SK.	N		M	Flowers of *Pulsatilla*
	Andrena sp.	AR Brooks	10 v 1950	Saskatoon SK.	N		M	*Salix* catkins
	Andrena sp.	LA Konotopetz	16 vi 1950	White Fox SK.	N		F	
	Andrena sp.	LA Konotopetz	14 vi 1950	White Fox SK.	N		F	
	Andrena sp.	NJ Atkinson	27 v 1925	Saskatoon SK.	N		M	
	Andrena sp.	AR Brooks	6 vi 1950	Saskatoon SK.	N		M	
	Andrena sp.	AR Brooks	6 vi 1950	Saskatoon SK.	N			
	Andrena sp.	AR Brooks	10 v 1950	Saskatoon SK.	N		M	*Salix* catkins
	Andrena sp.	AR Brooks	10 v 1950	Saskatoon SK.	N		M	*Salix* catkins
	Andrena sp.	LA Konotopetz	6 vi 1950	White Fox SK.	N		F	
	Andrena sp.	LA Konotopetz	14 vi 1950	White Fox SK.	N		F	Damaged left antenna
	Andrena sp.	LA Konotopetz	14 vi 1950	White Fox SK.	N		F	
	Andrena sp.	AR Brooks	12 v 1950	Saskatoon SK.	N		F	Damaged left antenna
	Andrena sp.	AR Brooks	11 vi 1950	Saskatoon SK.	N		F	Some pollen present
	Andrena sp.	AR Brooks	12 v 1950	Saskatoon SK.	N		F	Some pollen present
	Andrena sp.	AR Brooks	12 v 1950	Saskatoon SK.	N		F	
	Andrena sp.	AR Brooks	6 vi 1950	Saskatoon SK.	N		M	
	Andrena sp.	AR Brooks	6 vi 1950	Saskatoon SK.	N		M	Damaged right antenna

	Andrena sp.	AR Brooks	9 vi 1950	Saskatoon SK.	N		F	Small pollen load
	Andrena sp.	AR Brooks	12 v 1950	Saskatoon SK.	N		F	
	Andrena sp.	AR Brooks	9 vi 1950	Saskatoon SK.	N		F	
	Andrena sp.	AR Brooks	12 v 1950	Saskatoon SK.	N		M	
	Andrena sp.	AR Brooks	12 v 1950	Saskatoon SK.	N		M	
	Andrena sp.	AR Brooks	10 v 1950	Saskatoon SK.	N		M	
	Andrena sp.	AR Brooks	7 v 1950	Saskatoon SK.	N		M	Flowers of *Pulsatilla,* damaged right antenna
	Andrena sp.	AR Brooks	8 v 1950	Saskatoon SK.	N		M	*Cogswellia* flowers
	Andrena sp.	AR Brooks	10 v 1950	Saskatoon SK.	N		M	
	Andrena sp.	AR Brooks	10 v 1950	Saskatoon SK.	N		M	*Salix* catkins
	Andrena sp.	AR Brooks	7 v 1950	Saskatoon SK.	N		M	*P.ludovcia na* flowers
	Andrena sp.	AR Brooks	7 v 1950	Saskatoon SK.	N		M	*P.ludovcia na* flowers
	Andrena sp.	AR Brooks	7 v 1950	Saskatoon SK.	N		M	Flowers of *Pulsatilla*
	Andrena sp.	AR Brooks	12 v 1950	Saskatoon SK.	N		M	
	Andrena sp.	AR Brooks	12 v 1950	Saskatoon SK.	N		M	
	Andrena sp.	LA Konotopetz	25 v 1950	White Fox SK.	N		M	
	Andrena sp.	AR Brooks	12 v 1950	Saskatoon SK.	N		M	Damaged right antenna
	Andrena sp.	AR Brooks	12 v 1950	Saskatoon SK.	N		F	
	Andrena sp.	LA Konotopetz	25 v 1950	White Fox SK.	N		M	
	Andrena sp.	LA Konotopetz	25 v 1950	White Fox SK.	N		M	
	Andrena sp.	AR Brooks	8 v 1950	Saskatoon SK.	N		M	*Salix* catkins
	Andrena sp.	AR Brooks	8 v 1950	Saskatoon SK.	N		M	*Salix* catkins
	Andrena sp.	AR Brooks	12 v 1950	Saskatoon SK.	N		F	

	Andrena sp.	AR Brooks	12 v 1950	Saskatoon SK.	N		F	
	Andrena sp.	AR Brooks	12 v 1950	Saskatoon SK.	N		F	
	Andrena sp.	AR Brooks	12 v 1950	Saskatoon SK.	N		F	
	Andrena sp.	LA Konotopetz	3 vi 1950	White Fox SK.	N		F	
	Andrena sp.	LA Konotopetz	3 vi 1950	White Fox SK.	N		F	
	Andrena sp.	AR Brooks	25 v 1950	Saskatoon SK.	N		F	
	Andrena sp.	GE Fraser	July 1st 1949	Saskatoon SK.	N		F	
Sphecidae	*Crabro* sp.	A&J Brooks	22 viii 1959	White Fox SK.	N		F	
	Crabro sp.	A&J Brooks	18 viii 1958	Madge Lake SK.	N		F	
	Crabro sp.	A&J Brooks	22 viii 1959	White Fox SK.	N		F	
	Crabro sp.	A&J Brooks	23 vii 1958	Estevan SK.	N		M	
	Crabro sp.	A&J Brooks	23 vii 1959	Prince Albert SK.	N		M	
	Crabro sp.	A&J Brooks	17 viii 1958	Dauphin MB	N		M	
	Crabro sp.	A&J Brooks	23 vii 1959	Prince Albert SK.	N		M	
	Lindenius sp.	Brooks, McKay	13 vi 1957	Brooks Atla.	N		F	Damaged antenna
	Crossocerus sp.	JG Rempel	14 vi 1940	Regina SK.	N		M	
	Crossocerus sp.	?	12 ix 1947	Waterways	N		M	
	Crossocerus sp.	AH Sparrow	June 21st 1937	Swift Current SK.	N		F	
	Ectemnius sp.	S Morton	19 vi 1945	Saskatoon SK.	N		M	
	Ectemnius sp.	Brooks, Kelton	July 25th 1953	Horton MB.	N		F	
	Ectemnius sp.	?	4 ix 1947	Outpost	N		F	
	Ectemnius sp.	A&J Brooks	15 vii 1959	Christopher Lake SK.	N		F	

	Ectemnius sp.	Kenneth M. King	22 viii 1926	Marengo SK.	N		F	
	Ectemnius sp.	A&J Brooks	22 viii 1959	White Fox SK.	N		F	
	Ectemnius sp.	A&J Brooks	9/8 1959	Candle Lake	N		F	
	Ectemnius sp.	JS Thompson	July 21st 1938	Waskesiu	N		F	
	Ectemnius sp.	A&J Brooks	3 vii 1959	Big River SK.	N		F	Damaged right antenna
	Ectemnius sp.	?	25 viii 1947	Buffalo R.	N		F	
	Ectemnius sp.	?	1 vii 1940	Emma Lake	N		F	Damaged antennae
	Ectemnius sp.	?	12 ix 1947	Waterways	N		F	
	Ectemnius sp.	Anne Medhurst	August 8th 1965	Saskatoon SK.	N		F	
	Lestica sp.	?	22 vii 1940	Emma Lake	N		F	Sharing a pin.
	Lestica sp.	?	22 vii 1940	Emma Lake	N		M	Sharing a pin.
	Belomicrus sp.	LA Konotopetz	29 vii 1952	Elkwater Park Atla.	N		F	
	Belomicrus sp.	AR Brooks	14 vi 1956	Saskatoon SK.	N		F	
	Oxybelus sp.	?	8 viii 1947	Gros Cap?	N		F	
	Oxybelus sp.	?	4 vii 1944	Wallwart	N		M	
	Oxybelus sp.	AR&JE Brooks	21 viii 1957	Steveville Atla.	N		M	
	Oxybelus sp.	Brooks, Kelton	June 22nd 1953	Aweme MB	N		M	
Megachilidae	?	?	5 ix 1918	Saskatoon SK.	N		F	
	?	?	26 vi 1910	Regina SK.	N		F	
	?	AR Brooks	9 vi 1950	Saskatoon SK.	N		M	*Caragana*
	?	LG Saunders	August 22nd 1931	Montana	N		F	
	?	Bracken?	May 26th 1938	Saskatoon SK.	N		M	Damaged left antenna

	?	LG Saunders	August 17th 1931	Montana	N		F	Damaged left antenna
	?	?	July 27th 1938	Saskatoon SK.	N		F	Damaged anntenna
	?	LG Saunders	August 22nd 1931	Montana	N		F	Damaged right antenna
	?	AH Sparrow	June 21st 1937	Swift Current SK.	N		F	60 over '40-85?
	?	AR Brooks	9 vi 1950	Saskatoon SK.	N		M	*Caragana*
	?	JE McFarlane	Aug 19th 1949	Waskesiu	N		F	
	?	JS Thompson?	July 21st ?	Wask... SK.	N		F	
	?	?	Aug 1957	Brentwood BC	N		M	
	?	WB Fox	June 16th 1943	Elbow SK	N		F	98 over '40-85 Different family?
	?	LG Saunders	August 11th 1931	Montana	N		F	Damaged right antenna
	?	?	?	?	N		F	Damaged right antenna
	?	Bratten?	July 27th 1918?	S'K'toon	N		F	
	?	?	?	?	N		M	
	?	?	?	?	N		M	Damaged antennae
	?	E Evans	17 viii 1960	Athabasca SK.	Y	(1 mite-propodeum)	M	
	?	LG Saunders	August 18th 1931	Montana	Y	(1 mite prop)	F	
	?	?	?	?	Y	(1mite-ventral ab.)	M	Damaged antennae
	?	S Morton	July 20th 1943	Indian Head	Y	(1prop)(3 base R.M. Leg)(5 lft wng	F	

						bse)(5rt wng bse)		
	?	E Evans	21 viii 1960	Athabasca SK.	Y	(5prop-metasoma junction)	F	
	Megachile	JD Ritchie	27 vi 1941	Wallwort SK.	Y	(1 base prop)	F	*M. inermis* Damaged antennae
	?	?	?	?	N		F	Leaf case present [10]
	?	?	?	?	N		F	Leaf case present, damaged R antenna
	?	?	?	?	N		M	Leaf case present
	?	?	?	?	N		M	Leaf case present [10]
	?	?	?	?	N		F	Leaf case present [10]
	?	?	?	?	Y	(1 mite-placed on slide)	M	Leaf case present
	?	?	?	?	N		M	Leaf case present [10]
	?	?	14 vii 1947	Pearson Pt.	Y	(mite on Slide 4)	M	Damaged antennae
	?	Anne Medhurst	July 26th 1965	SW Calgary	N		F	Damaged left antenna
	?	E Evans	19 viii 1960	Athabasca SK.	N		F	
	?	E Evans	19 viii 1960	Athabasca SK.	N		F	
	?	E Evans	21 viii 1960	Athabasca SK.	N		F	
	?	?	54 ix 1916	Saskatoon SK.	N		F	
	?	M Erlandson	Sept. 11Th 1975	Saskatoon SK.	N		F	
Halictidae	?	LA Konotopetz	25 v 1950	White Fox SK.	N		F	
	?	LA Konotopetz	21 v 1950	White Fox SK.	N		F	

	?	AR Brooks	30 vi 1950	Saskatoon SK.	N		F	
	?	?	20 vi 1947	Resolution	N		F	
	?	AR Brooks	17 v 1950	Saskatoon SK.	N		F	
	?	LA Konotopetz	3 vi 1950	White Fox SK.	N		F	
	?	AR Brooks	25 v 1950	Saskatoon SK.	N		F	
	?	AR Brooks	11 vi 1950	Saskatoon SK.	N		F	
	?	AR Brooks	12 v 1950	Saskatoon SK.	N		F	
	?	LA Konotopetz	25 v 1950	White Fox SK.	N		F	
	?	LA Konotopetz	3 vi 1950	White Fox SK.	N		F	
	?	AR Brooks	12 v 1950	Saskatoon SK.	N		F	
	?	LA Konotopetz	3 vi 1950	White Fox SK.	N		F	
	?	LA Konotopetz	3 vi 1950	White Fox SK.	N		F	
	?	LA Konotopetz	25 v 1950	White Fox SK.	N		F	
	?	LA Konotopetz	21 v 1950	White Fox SK.	N		F	
	?	LA Konotopetz	21 v 1950	White Fox SK.	N		F	
	?	JE McFarlane	Aug. 1St 1949	Waskesiu SK.	N		F	
	?	E Sandercock	12 vi 1945	Indian Head	N		F	
	?	Kenneth M. King	Sept. 1St 1939	Saskatoon SK.	N		M	16423-1235 B 2nd Set
	?	Kenneth M. King	May 30th 1924	Saskatoon SK.	N		F	16425-4B6 87/'40-85
	?	Kenneth M. King	July 9th 1925	Saskatoon SK.	N		F	16446-170 BS
	?	?	July 16th 1938	Suth. Sask.	N		F	
	?	Kenneth M. King	May 27th 1924	Saskatoon SK.	N		F	16425-4e7

	?	S Morton	13 v 1944	Indian Head Sask.	N		F	
	?	E Sandercock	5 vi 1945	Indian Head	N		F	
	?	S Morton	9 vii 1944	Indian Head Sask.	N		F	
	?	E Sandercock	12 vi 1945	Indian Head	N		F	
	?	Smith	9 vi 1947	?	N		F	
	?	M. Cumming	6 viii 1945	Indian Head Sask.	N		M	Damaged antennae
	?	Briss...?	June 4th 1938	S'toon	N		F	
	?	Pearson	5 viii 1946	?	N		M	Damaged antennae
	?	Kenneth M. King	May 2nd 1924	Saskatoon SK.	N		F	16425-4e16 88/'40-85
	?	AR Brooks	11 vi 1950	Saskatoon SK.	N		F	
	?	AR Brooks	11 vi 1950	Saskatoon SK.	N		F	
	?	AR Brooks	11 vi 1950	Saskatoon SK.	N		F	
	?	S ?	July 31st 1938	Waskesiu SK.	N		M	
	?	?	30 viii 1947	Gros Cap?	N		M	
	?	E Sandercock	25 vi 1945	Indian Head	N		F	
	?	E McMillan	3 vi 1926	Saskatoon SK.	N		F	
	?	R Nursall	19-6-1946	Jasper Alta.	N		M	
	?	AR Brooks	25 v 1950	Saskatoon SK.	N		F	
	?	R Nursall	8-5-1946	Saskatoon	N		F	Missing most of its head
	?	?	17 vii 1947	Snowdrift NWT	N		M	
	?	JE McFarlane	27-01-49	Saskat	N		F	

	?	Kenneth M. King	May 30th 1924	Saskatoon SK.	N		F	16425-4B4 80/'40-85
	?	JST	May 7th 1940	S'toon	N		F	
	?	S Morton	8 vii 1944	Indian Head Sask.	N		F	
	?	?	June 12th 1938?	S'toon	N		F	
	?	ME Taylor	June 4th 1949	Love SK.	N		F	
	?	?	4 vii 1947	Wallwort	N		F	
	?	JG Rempel	5 viii 1944	Regina SK.	N		M	
	?	Pearson	15 viii 1947	?	N		F	
	?	S Morton	10 vii 1944	Neudorf, SK.	N		F	
	?	?	12 ix 1946	Kinley	N		F?	Missing antennae
	?	JE McFarlane	Aug 18th 1949	Waskesiu SK.	N		F	
	?	J McCown	30 v 1941	Saskatoon SK.	N		F	
	?	E Sandercock	5 vi 1945	Indian Head	N		F	
	?	Morton	July 17th 1943	Indian Head	N		M	
	?	JG Rempel	May 31st 1939	Waskesiu SK.	N		F	
	?	JG Rempel	May 26th 1939	Waskesiu SK.	N		F	
	?	JG Rempel	May 26th 1939	Waskesiu SK.	N		F	
	?	S Morton	22 ix 1944	Indian Head SK.	N		M?	Damaged antennae
	?	JG Rempel	May 29th 1939	Waskesiu SK.	N		F	

	?	?	29 ix 1946	Kinley	N		F	Head detached
	?	E Sandercock	12 vi 1945	Indian Head	Y	(3 mites on metasoma by propodeum)	F	
	?	JD Ritchie	6 vi 1945	Wallwort	Y	(1 mite right pleuron)(3 loose in hairs)	F	
	?	?	1 vi 1947	Waterways	Y	(1 loose at meta-prop junction)	F	
	?	R Nursall	19 6 1946	Jasper Alta.	N		M	(Halictidae ?)
	?	AE Cameron	18 vi 1918	Saskatoon	N		F	
	?	ET Reeder	May 30th 1949	Saskatoon SK.	N		M	(Halictidae ?)
	?	AR Brooks	12 v 1950	Saskatoon SK.	Y	(2 LHW)(1 RHW) (44 T1)(23 T2) (1 T3)	F	
	?	LA Konotopetz	12 vi 1950	Pas Trail SK.	Y	(7 RFW) (6 LHW) (4 LFW)	F	
	?	S Morton	1 ix 1944	Indian Head	N		M	
	?	R Nursall	19 6 1946	Jasper Alta.	Y	?	M	Slide 11 (Halictidae ?)
	?	?	5 ix 1918	Saskatoon SK.	N		M	(Halictidae ?)
	?	?	20 viii 1917	Saskatoon	N		F	(Halictidae ?)
	?	GE Fraser	July 1st 1949	Saskatoon SK.	N		F	Decapitated
	?	AR Brooks	11 vi 1950	Saskatoon SK.	N		F	Decapitated
	?	LA Konotopetz	25 v1950	White Fox	N		F	Missing abdomen
	?	AR Brooks	11 vi 1950	Saskatoon SK.	N		F	Decapitated

	Halictus sp.	JG Rempel	25 vi 1940	Big Muddy	N		F	
	Halictus sp.	JG Rempel	25 vi 1940	Big Muddy	N		F	
	Halictus sp.	JG Rempel	25 vi 1940	Big Muddy	N		F	
	Halictus sp.	?	26 ix 1942	Vernon BC	N		M	
	Halictus sp.	?	26 ix 1942	Vernon BC	N		M	
	Halictus sp.	JG Rempel	25 vi 1940	Big Muddy	N		F	
	Halictus sp.	E Cumming	5 vi 1946	Carlyle SK.	N		F	
	Halictus sp.	E Sandercock	19 vi 1945	Indian Head	N		F	
	Halictus sp.	?	26 ix 1942	Vernon BC	N		M	
	Halictus sp.	?	10 vi 1944	Swift Current SK.	N		F	
	Halictus sp.	BR Doig	Sept. 28th 1976	Saskatoon SK.	N		F	
	Halictus sp.	BR Doig	Sept. 28th 1976	Saskatoon SK.	N		F	
	Halictus sp.	BR Doig	Sept. 28th 1976	Saskatoon SK.	N		F	
	Halictus sp.	JG Rempel	25 vi 1940	Big Muddy	N		F	
	Halictus sp.	JG Rempel	25 vi 1940	Big Muddy	N		F	
	Halictus sp.	JG Rempel	25 vi 1940	Big Muddy	Y	?	F	Slides 2&3
	Halictus sp.	M Erlandson	Oct. 11Th 1975	Swanson SK.	N		M	
	Halictus sp.	DH Smith	Sept. 18Th 1969	Saskatoon	N		M	

Appendix Table 2. Bees collected during the 2013 field-season with mite distribution

Designation	Type of Bee	Male/Female	Mites?	Distribution/Description
6513#1	*Halictus rubicundus*	F	Y	RFW-16, RHW-14, LFW-25, LHW-20, Prop-7,Ab-met junc. 2, Thrx-10)
6513#2	*Halictus rubicundus*	F	Y	RFW-10, RHW-18, LFW-10, LHW-11
6513#3	*Halictus rubicundus*	F	Y	RFW-2, RHW-3, LFW-1, LHW-3
6513#4	*Halictus rubicundus*	F	Y	RFW-13, RHW-22, LFW-22, LHW-8, Prop-18, Ab-30, Thrx-6
6513#5	*Halictus rubicundus*	F	Y	RFW-15, RHW-24, LFW-15, LHW-30, Prop-23,T1-6
7513#5	*Lasioglossum*	F	Y	RHW-9,LHW-3
9513#4	*Lasioglossum*	F	N	
9513#5	*Halictus rubicundus*	F	Y	Prop-23, RHW-38, RFW-17, LHW-6, LFW-22, s1-2
9513#7	*Halictus rubicundus*	F	Y	T1-17, prop-20, thrx-3+3, base RFW-2, RFW-28, LFW-12, RHW-13, LHW-22
9513#8	*Lasioglossum*	F	N	
9513#9	*Lasioglossum*	F	N	
13513#1	*Halictus rubicundus*	F	Y	Prop-4, T1-1, RFW-8, RHW-25, LFW-12, LHW-21, S1-1, loose-1
13513#2	*Lasioglossum*	F	Y	LHW-1
13513#3	*Halictus rubicundus*	F	Y	LHW-36, LFW-15, prop-5, thrx-4, RHW-13, RFW-10, s1-2
13513#4	*Halictus rubicundus*	F	Y	thrx-6, prop-16, t1-1, rhleg-1, t2-1.t3-1, RHW-13, RFW-7, LHW-7, LFW-18
13513#5	*Halictidae*	F	N	
15513#4	*Halictidae*	F	Y	T1-28, t2-2
15513#7	*Halictus rubicundus*	F	Y	Prop-5, Rfw-15, rhw-20, lfw-14, lhw-24, s1-1
16513#2	*Halictidae*	F	N	
16513#6a	*Halictidae*	F	N	
16513#6b	*Halictidae*	F	N	
17513#1	*Halictidae-Lassioglossum*	F	N	
17513#2	*Halictidae-Lassioglossum*	F	Y	Lhw-2, rhw-4, t1-1, prop-possible mite, different from others,
17513#3	*Halictidae-Lassioglossum*	F	Y	T1-1, rfw-1,lfw-1, possible mite (1), between coxae (different from other mites present)
17513#4	*Halictidae-Lassioglossum*	F	Y	T1-16, rhw-1, lhw-3,
17513#6	*Halictidae-Lassioglossum*	F	N	
21513#5	*Halictidae-Lassioglossum*	F	N	
21513#6	*Halictidae-Lassioglossum*	F	Y	Lhw-1,
22513#2	*Halictidae-Lassioglossum*	F	N	
275#3	*Halictidae-Sphecodes*	M	N	
295#6	*Halictidae-Halictus*	F	N	
295#7	*Halictidae-Lassioglossum*	F	N	
3613#3	*Halictidae-Lassioglossum*	F	N	
6613#4	*Sphecodes?*	M	N	
6613#5	*Halictidae-Lassioglossum*	F	N	
6613#6	*Halictidae-Lassioglossum*	F	N	
11613#2	*Halictidae-Lassioglossum*	F	N	
11613#3	*Halictidae-Lassioglossum*	F	N	
12613#1	*Halictidae-Lassioglossum*	F	N	

17613#4	*Lasioglossum*	F	N	
17613#8	*Lasioglossum*	F	N	
18613#3	*Halictidae-Halictus confusus*	F	Y	T1-13, t2-3, t3-2, rfw-3, rhw-2, lfw-3, lhw-2
18613#7	*Lasioglossum*	F	N	
18613#10	*Lasioglossum*	F	N	
246#1	*Lasioglossum*	F	N	
246#2	*Lasioglossum*	F	N	
246#7	*Lasioglossum*	F	N	
246#13	*Lasioglossum*	F	N	
266#1	*Lasioglossum*	F	N	
27613#5	*Lasioglossum*	F	N	
27613#9	*Lasioglossum*	F	Y	Rhw-1, lfw-1
28613#5	*Lasioglossum*	F	N	
2713#1	*Lasioglossum*	F	N	
2713#2	*Lasioglossum*	F	N	
2713#4	*Halictus rubicundus*	F	Y	Rhw-12, lhw-8, lfw-5, rfw-16
2713#5	*Halictus rubicundus*	F	Y	Prop-9, loose hairs of left side prop-1, s1-1, t1-4, lhw-16, lfw-12, rhw-24, rfw-11
2713#6	*Lasioglossum*	F	N	
2713#8	*Lasioglossum*	F	N	
4713#1	*Lasioglossum*	F	N	
4713#2	*Halictus rubicundus*	F	Y	Prop-3, thrx-1, lfw-29, lhw-34, rfw-25, rhw-39, s1-1
8713#1	*Lasioglossum*	F	N	
8713#4	*Lasioglossum*	F	N	
8713#9	*Lasioglossum*	F	N	
8713#13	*Halictus*	F	N	
9713#2	*Halictus rubicundus*	F	Y	Rhw-2, rfw-5, lhw-4, lfw-1
9713#4	*Lasioglossum*	F	N	
9713#11	*Lasioglossum*	F	N	
11713#8	*Lasioglossum*	F	N	
11713#11	*Lasioglossum*	F	N	
11713#12	*Halictus*	F	Y	(LFW-2)(RFW-2)(RHW-8)(T1-48)
11713#13a	*Sphecodes*	M	N	
11713#13b	*Sphecodes*	M	N	
11713#13c	*Sphecodes*	M	N	
15713#4	*Lasioglossum*	F	N	
15713#9	*Lasioglossum*	F	N	
15713#11	*Lasioglossum*	F	N	
15713#13	*Lasioglossum*	F	N	
16713#2	*Lasioglossum*	F	N	
16713#7	*Lasioglossum*	F	N	
16713#8	*Lasioglossum*	F	N	
17713#1	*Halictus*	F	Y	(RFW-2)
17713#4	*Sphecodes?*	M	N	
17713#8	*Lasioglossum*	F	N	
18713#4	*Lasioglossum*	F	N	
19713#5	*Lasioglossum*	F	N	
19713#8	*Lasioglossum?*	F	N	

19713#9	*Halictus rubicundus*	F	N	
19713#10	*Halictidae*	M	N	
237#5	*Halictus rubicundus*	F	Y	(LHW-1)
28#2	*Halictus rubicundus*	F	Y	(S1-3)(RHW-25)(T1-18)(Prop-27)(thrx-11)(LHW-17)(LHW-30)(RFW-21)(loose in ventral hairs-3)
28#5	*Halictus rubicundus*	?	N	
28#7	*Halictus rubicundus*	M	Y	(LHW-1)
128#8	Halictidae	M	N	
128#9	Halictidae	M	N	
128#11	Halictidae	M	N	
128#13	Halictidae	M	N	
128#14	Halictidae	M	N	
138#3	Halictidae	F	Y	(t1-2)(RFW-1)(LHW-2)(RHW-5)(LFW-2)
225#3	*Halictus rubicundus*	F	Y	(RFW-1)(LHW-1)
9513#1	*Megachilidae-Osmia*	M	N	
5613#1	*Megachilidae-Megachile*	F	N	
7613#3	*Megachilidae-Megachile*	F	N	
7613#4	*Megachilidae-Megachile*	F	N	
7613#5	*Megachilidae-Megachile*	M	Y	Prop-1, L fore coxa/leg-2, R fore coxa/leg-2
17613#3	*Megachile*	M	N	
17613#9	*Megachile*	F	N	
18613#9	*Megachile*	F	Y	S1-3, lhleg base-1
246#6	*Megachile*	M	Y	rforeleg base-9, lforeleg base-6, rhcoxa base-1, lhind coxa-1, possible mite-s1
246#9	*Megachile*	M	Y	Met-thrx junc.-1, r eye-1, l eye-1, left mandible-1, rfemur-1, base of right leg-2, genitals-2
246#10	*Coelioxys*	M	N	
246#11	*Megachile*	F	Y	t1-15, prop-5(thrx,base lwings-3),(t1 hairs loose-2), loose-hairs of mid l leg-2, r mid leg-2
246#12	*Megachile*	F	Y	loose on hairs back left coxa-1, loose hairs ventral thrx-1, rforeleg base-1, loose hairs of l legs
266#3	*Megachile*	M	N	
266#4	*Hoplitis*	M	N	
26613#8	*Megachile*	M	N	
27613#1	*Megachile*	M	N	
27613#3	*Megachile*	M	N	
27613#4	*Heriades*	M	N	
27613#6	*Megachile*	M	Y	Met-ab junction-1, lforeleg base-12, r foreleg base-13, lmid leg-2, s1-1, rmid-4, rhind leg-1
27613#11	*Coelioxys*	F	N	
27613#12	*Hoplitis*	F	N	
27613#14	*Megachile*	M	N	
27613#15	*Megachile frigida*	M	N	
28613#3	*Megachile*	M	Y	rmiddle leg base-1, lforeleg-2, rforeleg-1, fore coxae-4
28613#7	*Megachile rotundata*	M	N	
2713#3	*Megachile*	M	N	
2713#7	*Coelioxys*	M	N	

2713#12	*Megachile frigida*	M	Y	rfore coxa-3
8713#2	*Stelis*	M	N	
8713#3	*Heriades*	M	N	
8713#6a	*Megachile*	M	N	
8713#12	*Megachile*	F	N	
9713#5	*Heriades*	M	N	
9713#6a	*Megachile*	F	N	
11713#6	*Megachile*	M	N	
11713#9	*Megachile*	M	N	
11713#10	*Megachile latimanus*?	M	N	
15713#1	*Megachile*	M	N	
16713#9	*Megachile*	F	N	
17713#2	*Megachile*	M	N	
17713#3	*Megachile*	?	N	
17713#9b	*Megachile*	F	N	
18713#5	*Hoplitis?*	F	N	
19713#2	*Megachile*	M	N	
19713#4	*Megachile*	M	N	
317#6	*Megachile*	M	N	
28#1	*Megachile*	F	N	
28#6	*Megachile*	F	N	
128#3	*Megachile*	F	N	
128#5	*Megachile*	F	N	
128#7	*Megachile*	M	N	
7513#2	*Andrena*	M?	N	
7513#3	*Andrena*	F	Strepsiptera	Between 4th and 5th tergites
9513#10	Andrenidae-*Andrena*	M	N	
9513#12	Andrenidae-*Andrena*	F	N	
14513#2	*Andrenidae*	M	N	
15513#1	*Andrenidae*	M	N	
15513#2	*Andrenidae*	M	N	
16513#1	*Andrenidae*	M	N	
16513#3	*Andrenidae*	M	N	
16513#4	*Andrenidae*	M	Y	Hairs of thrx-2, T1-1
16513#5	*Andrenidae*	M	N	
16513#7	*Andrenidae*	M	N	
17513#5	*Andrenidae-Andrena*	M	N	
17513#7	*Andrenidae*	M	N	
21513#1	*Andrenidae-Andrena*	F	N	
21513#2	*Andrenidae-Andrena*	F	Strepsiptera	Between 4th and 5th tergites
21513#4	*Andrenidae-Andrena*	F	N	
225#1	*Andrena*	F	N	
225#4	*Andrenidae-Andrena*	F	N	
225#5	*Andrenidae-Andrena*	F	N	
225#6	*Andrenidae-Andrena*	F	N	
225#7	*Andrenidae-Andrena*	F	Y	Loosely attached to hair on leg-1
235#2	*Andrenidae-Andrena*	F	N	
235#4a	*Andrenidae-Andrena*	M	N	

235#4b	*Andrenidae-Andrena*	M	N	
235#4c	*Andrenidae-Andrena*	M	N	
275#1	*Andrenidae-Andrena*	F	N	
275#2	*Andrenidae-Andrena*	F	N	
275#4	*Andrenidae-Andrena*	M	N	
275#5	*Andrenidae-Andrena*	F	N	
295#2	*Andrenidae-Andrena*	M	N	
295#3	*Andrenidae-Andrena*	F	N	
295#4	*Andrenidae-Andrena*	F	N	
295#5	*Andrenidae-Andrena*	M	N	
305#4	*Andrenidae-Andrena*	M	N	
305#7	*Andrenidae-Andrena*	F	N	
305#8	*Andrenidae-Andrena*	M	N	
315#1	*Andrenidae-Andrena*	F	N	
315#2	*Andrenidae-Andrena*	M	N	
315#3	*Andrenidae-Andrena*	F	N	
3613#2	*Andrenidae-Andrena*	M	N	
3613#4	*Andrenidae-Andrena*	F	N	
5613#5	*Andrenidae-Andrena*	F	N	
5613#8	*Andrenidae-Andrena*	F	N	
5613#10	*Andrenidae-Andrena*	F	N	
5613#11	*Andrenidae-Andrena*	F	N	
5613#12	*Andrenidae-Andrena*	F	N	
5613#14	*Andrenidae-Andrena*	F	N	
5613#15	*Andrenidae-Andrena*	F	N	
5613#16	*Andrenidae-Andrena*	F	N	
6613#2	*Andrenidae-Andrena*	F	Strepsiptera	
6613#3	*Andrenidae-Andrena*	F	N	
11613#5	*Andrenidae-Andrena*	M	N	
11613#6	*Andrenidae-Andrena*	F	N	
11613#8	*Andrenidae-Andrena*	F	N	
11613#9	*Andrenidae-Andrena*	F	Strepsiptera	4th and 5th
12613#2	*Andrenidae-Andrena*	F	Strepsiptera	4th and 5th
12613#3	*Andrenidae-Andrena*	F	N	
12613#4	*Andrenidae-Andrena*	F	Strepsiptera	4th and 5th
18613#1	*Andrena*	F	N	
18613#4	*Andrena*	F	N	
18613#5	*Andrena*	F	N	
18613#6	*Andrena*	F	N	
266#5	*Andrena*	F	N	
4715#8	*Andrena*	F	N	
15713#5	*Andrena*	F	N	
18713#1	*Andrena*	M	N	
19713#6	*Andrena*	F	N	
19713#11	*Andrena*	M	N	
225#7	*Andrenidae*	F	N	
6513#6	*Apis mellifera*	F	N	
6513#7	*Apis mellifera*	F	N	

6513#8	*Apis mellifera*	F	N	
7513#1	*Apis mellifera*	F	N	
9513#2	*Apis mellifera*	F	N	
9513#11	*Nomada*	M	N	
21513#3	*Apis mellifera*	F	N	Strepsiptera larvae in pollen load
235#3	*Apidae-Nomada*	?	N	
305#3	*Apidae-Nomada*	F	N	
305#5	*Apidae-Nomada*	M	N	
5613#2	*Apidae-Nomada*	F	N	
5613#7	*Apis mellifera*	F	N	
17613#2	*Apis mellifera*	F	N	
17613#10	*Nomada*	M	N	
27613#10	*Nomada*	F	N	
4713#3	*Apis mellifera*	F	N	
4713#5b	*Apis mellifera*	F	N	
11713#4	*Anthophora*	M	N	
15713#14	*Anthophora*	M	N	
237#9	*Martinapis or Peponapsis?*	F	N	
237#10	*Apis mellifera*	F	N	
7513#4	*Bombus*	F	Y	abdomen hairs, loosely attached-3
9513#3	*Bombus*	F	Y	Abdomen(T1-T2)-1
14513#1	*Bombus*	F	Y	?-many, but obscured by hair, left side thrx-37, right side thrx-35, Abdomen (T2/T3-?15)
235#1	*Bombus*	F	Y/N	1-Removed, loose on hairs
305#2	*Bombus*	F	N	
7613#1	*Bombus*	F	N	
7613#6	*Bombus*	F	Y	
11613#1	*Bombus*	F	N	
12613#5	*Bombus*	F	N	
17613#1	*Bombus*	F	N	
17613#5	*Bombus*	F	N	
17613#6	*Bombus*	F	N	
17613#7	*Bombus*	F	N	
18613#2	*Bombus*	F	Y	One large mite, many smaller attached
19613#2	*Bombus*	F	N	
19613#4	*Bombus*	F	N	
246#3	*Bombus*	F	N	
246#4	*Bombus*	F	N	
246#8	*Bombus*	F	N	
246#14	*Bombus*	F	N	
28613#1	*Bombus*	F	N	
28613#2	*Bombus*	F	N	
28613#6	*Bombus*	F	N	
2713#13	*Bombus*	M	N	
4713#4	*Bombus*	F	N	
4714#6	*Bombus*	F	N	
8713#5	*Bombus*	F	N	
8713#11	*Bombus*	F	N	

9713#1	*Bombus*	F	N	
9713#10	*Bombus*	F	N	
9713#13	*Bombus*	F	N	
15713#10	*Bombus*	F	N	
15713#12	*Bombus*	F	N	
16713#6	*Bombus*	F	N	
17713#5	*Bombus*	F	N	
17713#6	*Bombus*	F	N	
17713#7	*Bombus*	F	N	
18713#2	*Bombus*	F	N	
18713#3	*Bombus*	F	N	
19713#1	*Bombus*	F	N	
19713#3	*Bombus*	F	N	
237#1	*Bombus*	M	Y	(T2-11)(T3-8)(T4-5)
237#6	*Bombus*	F	N	
237#7	*Bombus*	F	N	
247#6	*Bombus*	F	N	
247#8	*Bombus*	F	N	
247#9	*Bombus*	F	N	
317#3	*Bombus*	F	N	
317#5	*Bombus*	F	Y	(T2-1)
317#12	*Bombus*	F	N	
28#3	*Bombus*	F	N	
28#4	*Bombus*	F	N	
128#2	*Bombus*	F	N	
128#4	*Bombus*	M	N	
128#6	*Bombus*	F	Y	(T5-1)(loose in ventral hairs-1)
138#2	*Bombus*	M	N	
138#7	*Bombus*	F	N	
26613#9	*Hylaeus*	M	N	
26613#10	*Hylaeus*	M	N	
27613#2	*Hylaeus*	M	N	
27613#7	*Hylaeus*	M	N	
27613#13	*Hylaeus*	M	N	
8713#6b	*Hylaeus*	F	N	
11713#2	*Hylaeus*	F	N	
11713#7	*Hylaeus*	F	N	
18613#8	*Colletidae?*	M	Y	Venter S1
247#4	*Colletidae*	F	N	
4713#5a	?	F	N	
247#5	?	?	N	

Appendix Table 3. Bees caught during the preparatory phase of 2014.

Designation	Type of Bee	Male or Female	Mites ?	Distribution/Description
NS-14#1	Halictidae	F	Y	(thrx-14)(prop-1)(LHW-14)(RHW-25)(RFW-30)(LFW-8)
NS-14#2	Andrenidae	M	N	
NS-14#3	Halictidae	F	Y	(T1-27)(prop-14)(RHW-10)(RFW-16)(LHW-24)(LFW-14)
NS-14#4	Andrenidae	M	N	
NS-14#5	Andrenidae	M	N	
NS-14#6	Andrenidae	M	N	
NS-14#7	Halictidae	F	N	
NS-14#8	Andrenidae	M	N	
NS-14#9	Andrenidae	M	N	
NS-14#10	Andrenidae	F	N	
NS-14#11	*Halictus*	F	Y	(T1-2)(prop-9)(LHW-30)(LFW-21)(RHW-26)(RFW-20)
NS-14#12	Halictidae	F	N	
NS-14#13	Halictidae	F	N	
NS-14#14	Halictidae	F	N	
NS-14#15	Andrenidae	M	N	
130514#1	Andrenidae	M	N	
130514#2	Andrenidae	M	N	
130514#3	Andrenidae	M	N	
130514#4	Halictidae	F	Y	(prop-1)(T1-1)(RFW-3)(RHW-6)(LFW-6)(LHW-2)(loose on ventral hairs-2)
130514#5	Andrenidae	M	N	
140514#1	Andrenidae	M	N	
140514#2	Andrenidae	M	N	
140514#3	Andrenidae	M	N	
140514#4	Andrenidae	M	N	
140514#5	Andrenidae	F	N	
140514#6	Andrenidae	F	N	
140514#7	Andrenidae	M	N	
140514#8	Andrenidae	M	N	
140514#9	Andrenidae	M	N	
140514#10	Andrenidae	M	N	
140514#11	Andrenidae	M	N	
140514#12	Andrenidae	M	N	
140514#13	Andrenidae	F	N	
140514#14	Andrenidae	M	N	Strepsiptera
140514#15	Andrenidae	M	N	
2705#1a	Andrenidae	M	N	
2705#1b	Andrenidae	M	N	
2705#1c	Andrenidae	M	N	
2705#2	Andrenidae	F	N	
2705#4	Andrenidae	F	N	
2705#5	Andrenidae	F	N	

2705#6	Andrenidae	F	N	
2705#7	Andrenidae	F	N	
2705#8	Andrenidae	M	N	
2705#9	Andrenidae	F	N	
2705#10	Andrenidae	F	N	

Appendix Table 4. Bees caught when excavating during 2014.

Designation	M/F	Mites?	Quantity and distribution	Mature/ Immature	Notes
N1 Achilt	F	Y	(RFW 17) (RHW19) (LFW 16) (LHW 15)	Mature	
Bus T	F	Y	(RHW 22) (LHW 8)	Mature	
208#2	F	Y	(prop 1) (RHW 7) (LHW 1)	Mature	
2014 Im #1	M	Y	(Prop 32)(RW 4)(LW 2)(Head 1)(LBack&LMid leg base 1)	Immature	
NS #3	F	Y	(R thrx 11) (L thrx 9)	Immature	
2014 Male #1	M	Y	(1 between mid coxae)	Mature	
Bus T	F	Y	(LFW 3)(RFW 4)(LHW 47)(RHW 27)(thrx 1)	Mature	
N5	M	Y	(Thrx 7) (L ante. 1)	Immature	
Hal #1	F	Y	(T1 37)(prop 16)(RFW 25)(RHW 14)(LFW 29)(LHW 21)(Lwbs4)	Mature	
N5	F	Y	(sternite1 3)(RHW 9)(RFW 4)(LHW 7)(RFW 6)	Mature	
Hal #2	F	Y	(LHW 3)(LFW 2)(RFW 3)(RHW 2)(prop 15)(thrx L&R 2)	Mature	
Bus T 3814	M	Y	(thrx. 4)(Lthorax 2)(Rthorax8)(LW 1)(Fcoxa 1&1)(Head 4)(leg 1)	Immature	
Bus T 7814	F	Y	(t1 16)(prop 39)(RFW 13)(RHW 8)(LFW 21)(LHW 18)(thrx. 5)	Mature	
2014 Im #2	F	Y	(Rprop 4)(RW 6)(RL 4) (R ante. 1)(L thorax 13)(LW 2)(Lleg 1)	Immature	
N? 09/5	F	Y	(Rthorax 10)(RW 5)(Lthorax 19)(backthorax 4)(LW 7)	Immature	
20/8#7	M	Y	(ventral thrx btwn 2nd3rd coxae 7)(Ventral head,right of labrum 5)	Mature	
N1 7/7 2014	F	Y	(left hind leg, near abdomen 2?)(Ventral-rightwing1,abdomen3, -lefthind femur)	Immature-nearly mature	Obscured
20/8#1	M	Y	(ventral btwn 2nd & 3rd coxae 8)	Mature	
Bus T 7814-1	F	Y	(thorax/prop 38)(right hind leg 2)(right wing 7+7)(left wing 7+13)(Lthrx6)(Rthrx8)	Immature(Nearly mature)	
2014 Im#3	F	N?	(1? on thorax)	Immature?-desiccated	Fungal growth
20/8#3	F	Y	(right forewing19)(Rhind wing11)(Lforewing 12)(Lhindwing 14)(prop+thrx=38+2)	Mature	
Hal #2	F	Y	(Lhindwing 3)(Rforewing 4+2)(Lforewing 2)(prop 5)	Just pupated?	
N2 -2	M	Y	(Rforewing 1)(R hindwing 4)(L forewing 4)(Right middle leg tibia 1)	Mature	

Hal #3	F	Y	(Lforewing 3)(Lhindwing 17)(Rforewing 5)(Rhindwing 11)	Mature	
Bus T 7814-2	F	Y	(prop 3)(Lhindwing 1)(Lforewing 6)(R forewing 7)(Rhindwing 8)	Mature	
N5	F	Y	(Rhindwing 16)(Rforewing 6)(ventral abdomen S1-6)(L-9)(LHW 6)(prop 10)	Mature	
2014 Im #4	F	Y	(thrx 8)(rw 1)	Immature	
3	F	N?		Mature?	Fungal growth
N2 1	M	Y	(thrx 12)(lhindfemur 2)(rw 3)(under lw 1)	Immature	
N4	F	Y	(lw 12)(thrx/prop 27)	Immature	
N5	F	Y	(prop 3)(Lhindwing 29)(lforewing 12)(Rfw 1)(fhw 14)	Mature	
Hal #4	F	Y	(lhw 7)(lfw 3)(rfw 2)(rhw 7)(prop 8)	Mature	
Hal #5	F	Y	(T1- 3)(thrx-2)(prop-2)(lhw 1)(rfw 1)(rhw 1)	Mature	
Hal Larva	?	N		Immature	Larva